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THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XIX SEPTEMBER, 1943 NUMBER 5

EARLY LESIONS OF EXPERIMENTAL ENDOCARDITIS LENTA *

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The transmission of infection with *Streptococcus viridans* by intravenous injection of pure cultures into animals with the production of an experimental disease closely resembling the human endocarditis due to infection with this organism has been previously reported.[1] By the simple procedure of intravenous inoculation one may cause endocardial vegetations in various animals such as rabbits, rats and mice. Rabbits, because of their conveniently large ear veins, have been used by us for the most part. It has seemed best to designate this experimental infectious disease, which may be regarded as specific in the bacteriologic sense, as experimental endocarditis lenta. Our collection of material from these experimental animals has been studied in some detail in order to follow the sequence of events in the experimental disease. Photographs of the fully developed valvular vegetation were shown in the previous paper. In this present communication we purpose to deal with the changes observed in the heart in the earlier stages of the experimental disease, where the lesions are, for the most part, so minute as to be readily overlooked in gross inspection of the heart.

Rabbit 36 was inoculated by intravenous injection of 2 to 4 cc. of a culture suspension of *Streptococcus viridans*, strain P, that had been centrifugalized, washed in saline solution, recentrifugalized and suspended in saline solution, daily on May 10, 11, 12 and 13, 1939. The rabbit died early on May 15th. At autopsy there were visible many small renal abscesses and small rough spots on the tricuspid valve, thought to be early vegetations. Sections through this region actually showed definite early vegetations near the margin of one of the tri-

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cuspid flaps. The earliest changes, however, were seen in the endocardial endothelium of the ventricle and in the myocardial capillaries. Figure 1 represents a small portion of the right ventricle and Figure 3 is a drawing of a small area of this section. In almost every microscopic field one can find streptococci contained within endocardial endothelial cells. Bacteria are few and the endothelial cells seem in many instances to be free from other alteration. Some of them, however, are thickened and partly loosened. Sometimes the nucleus appears more rounded than normally or may even exhibit irregular fragmentation of the chromatin. At one place about midway in the figure there is some material adherent to the endocardium and in this a few erythrocytes can be distinguished. The subjacent myocardium appears unaltered, but in it near the center of the drawing there is a capillary almost filled with a thrombus, containing many streptococci, most of them within polynuclear leukocytes. There is a slight excess of wandering cells external to the capillary wall and, at a short distance to the left in the drawing, there are extravasated erythrocytes. At the bottom of the drawing there are represented four serial sections of this thrombosed capillary, the third in the series belonging to the section represented in the main drawing. Several normal capillaries may be seen, two fairly large ones at the upper right.

Rabbit 34 was inoculated by intravenous injection of 2 to 4 cc. of *Streptococcus viridans*, strain P, that had been washed and then suspended in saline solution, daily on May 10, 11 and 12, 1939. Blood culture taken on May 15th was positive. The rabbit died on the morning of May 16th. At necropsy there was seen a small irregular thickening near the margin of a mitral flap. Sections through this leaflet actually revealed an irregular accumulation of fibrin near the free edge and there were many well stained cocci contained in this fibrin. On examining the section of the leaflet nearer to its base there was seen on the auricular surface an irregular displacement of the endothelial cells in which a few streptococci could be distinguished even in the mihroscopic section stained with hematoxylin and eosin (Fig. 4). Erythrocytes also were recognized along with amorphous material, apparently fibrin and platelets, adherent to this auricular endothelial surface. Another section of this same mitral flap, at some distance from the first section, stained by the method of Brown and Brenn,[2] revealed the streptococci more distinctly (Fig. 7). Here also there was distortion of the endothelial cells and of their nuclei, and a small thrombus, containing several erythrocytes, was adherent over an area almost 0.1 mm. in extent. In both drawings the subendothelial edema is distinctly shown and it is worthy of note that the endothelium

on the ventricular surface seems to have escaped alteration to a very large extent. Careful study of several sections failed to disclose any cocci included by phagocytosis in the endothelium on the ventricular surface of the mitral leaflet.

Rabbit 33, weighing 2,000 gm., was inoculated by intravenous injection of 2 cc. of culture no. 353 daily on May 10, 11, 12 and 13, 1939. There was no further inoculation. Blood culture taken on May 15th gave positive growth, as also did a second one taken on May 18th. The animal died at 2:30 p.m., May 19th, or 9 days after the initial inoculation. At autopsy there were gross infarcts in both kidneys and minute vegetations were recognized on the mural endocardium of the right ventricle and of the left ventricle as well as on the mitral and aortic valves. The liver showed considerable coccidiosis.

Figure 2 shows a photomicrograph at low magnification representing a section passing through the left ventricle, an aortic cusp and a portion of the aortic wall. The finer details are illustrated by the colored drawings. In Figure 5 there is pictured a small bit of the ventricular endocardium. The endothelial cells contain engulfed streptococci but seem not to be greatly altered. A capillary just beneath the endocardium is cut longitudinally and appears to be normal. The myocardial cells, however, are separated by edema fluid and are also somewhat fragmented. The endothelial lining of the ascending aorta, shown in Figure 8, also contains many cocci and, for the most part, is without marked alteration of the endothelium or the subjacent stroma. However, over an area of about 0.5 mm. in length the endothelial cells have been destroyed by the massive proliferation of the bacteria and the underlying stroma has been invaded by streptococci with the production of edema and, in places, coagulation necrosis. Over these latter, more severe lesions, the bacteria are seen as compact masses of cocci directly in contact with the fluid within the aortic lumen.

A photomicrograph of the section of the aortic cusp is shown in Figure 10 and more detail is shown in the colored drawings. Figure 6 represents the entire thickness of the valve. On the aortic side one recognizes the scattered cocci adherent to, and included by phagocytosis in the endothelial cells, without much evident alteration of these latter elements. On the ventricular face of the valve, on the other hand, the endothelial cells contain many more bacteria. The endothelial cells themselves are swollen and are elevated irregularly by the accumulation of edema fluid in the subjacent stroma. In places there are small amounts of fibrin adherent to the free surface and these deposits sometimes contain recognizable erythrocytes. Such a small deposit is shown near the upper left corner of the drawing. Figure

13 represents a portion of the same aortic leaflet and shows the ventricular face at the site of the curve or kink, readily identifiable in the photomicrographs. Here phagocytosis of the cocci is also evident and at one place, at the right, a polynuclear leukocyte containing cocci is adhering to the endothelium. Of particular interest, however, is the lesion at the left of the figure, where the streptococci have already multiplied to form a dense bacterial colony with more or less advanced destruction of the endothelial cells. An irregular deposit of fibrin with incarcerated erythrocytes is attached to this altered surface and constitutes a minute, irregular projection which was grossly recognizable as an early vegetation. This is large enough to be seen in many serial sections.

In the sections of this heart there are also lesions in the myocardial vessels. Figure 9 represents a coronary arterial branch of a diameter of about 0.4 mm. and the interesting portion of the arterial wall is shown in Figure 11. At one place the endothelial cells of the intima have engulfed some of the streptococci and here there is a small adherent thrombus, evidently composed of fibrin, platelets and incorporated erythrocytes, and including easily recognizable streptococci. This lesion is evidently similar to those seen in the ascending aorta of this same animal. Figure 12 shows a thrombosed capillary in the ventricular myocardium. The thrombus extends along the capillary and contains polynuclear leukocytes and masses of bacteria toward the upper end in the picture. Toward the bottom of the figure the clot consists chiefly of fibrin and altered erythrocytes without visible cocci, suggesting an extension of the clot by the process of relatively aseptic thrombosis. This would appear to be a rather recent obstruction because of the slight changes in the adjacent myocardium. However, for decision in regard to mode of origin, even this vascular lesion is already too far advanced. Whether it started by endothelial phagocytosis of circulating streptococci or by the attachment of a polynuclear leukocyte already loaded with cocci remains uncertain but the latter mode of origin seems probable.

Discussion

The significance of these observations in relation to the sequence of events in the development of the lesions of endocarditis requires little discussion because the evidence is in itself so clear. Obviously, an endocardial lesion might develop by extension of the inflammatory process from a thrombosed capillary near the endocardial surface, such as that shown in Figure 3 (rabbit 36). This might easily be accepted as the mode of origin of some mural vegetations. In our experimental

rabbits, however, such thrombosed capillaries are found after considerable search, whereas the endothelial cells of the mural endocardium may everywhere serve as phagocytes for cocci. It seems, therefore, that the great majority of the valvular and mural lesions as well as those of the aortic and arterial walls take origin from the intimal implantations of the circulating bacteria. Subsequently the endothelial cells may destroy the included cocci so that the lesion heals without residual damage, and this evidently takes place over considerable areas of the endocardium, as will be evident in the study of later stages of this disease. Failing this, the lesion may progress to cause more serious structural alteration and functional incapacity. This failure to heal evidently depends upon several factors: first, the virulence of the infecting bacteria; second, the general resistance of the host; third, other miscellaneous circumstances. Without entering too fully into these questions at the moment, we may here point out the contrast between the two surfaces of a heart valve. As may be seen in the drawings, it is the auricular surface of the mitral valve and the ventricular surface of the aortic valve which seem more favorable to the proliferation of the bacteria. This, we believe, is due to the greater physical trauma to which these surfaces are exposed. Each mitral leaflet comes into contact with its fellow at each systole of the ventricle so that the endothelial cells on the auricular surfaces over the area of this contact are closely applied to each other and they are peeled apart at each ventricular diastole. Similar contact under pressure affects the ventricular surfaces of the aortic leaflets. Physical relationships of pressure, speed of blood flow, stagnation of the blood and contact with other solid elements doubtless also play a part in the relative frequency of lesions in the ascending aorta, sinus of Valsalva, arteries, capillaries and veins, but the present experimental material does not warrant any extended discussion of these relationships.

Summary

1. Following the intravenous injection of large amounts of washed bacteria as well as untreated cultures of *Streptococcus viridans* into rabbits, the bacteria are taken up extensively by phagocytosis by the endothelial cells of the endocardium and of the intima of the aorta and coronary arteries.

2. The bacteria also lodge in the myocardial capillaries either by direct endothelial phagocytosis of streptococci or by arrest of sluggish leukocytes containing the bacteria.

3. After phagocytosis many bacteria are evidently destroyed without production of recognizable persistent structural changes.

4. In some places, particularly on the heart valves, the included bacteria tend to survive, multiply, and initiate the precipitation of elements from the blood so as to give rise to the vegetations of endocarditis.

5. Local physical factors play some part in the progress of these local lesions.

This paper is a report of the results of joint effort. The experimental work on the animals was performed for the most part by Miss Spence. The microscopic sections were prepared by Miss Slavkin. The illustrations in color were drawn by Dr. MacNeal.

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1. MacNeal, W. J., Spence, M. J., and Wasseen, M. Experimental production of endocarditis lenta. *Am. J. Path.*, 1939, **15**, 695–705.
2. Brown, J. H., and Brenn, L. A method for the differential staining of Gram-positive and Gram-negative bacteria in tissue sections. *Bull. Johns Hopkins Hosp.*, 1931, **48**, 69–73.

DESCRIPTION OF PLATES

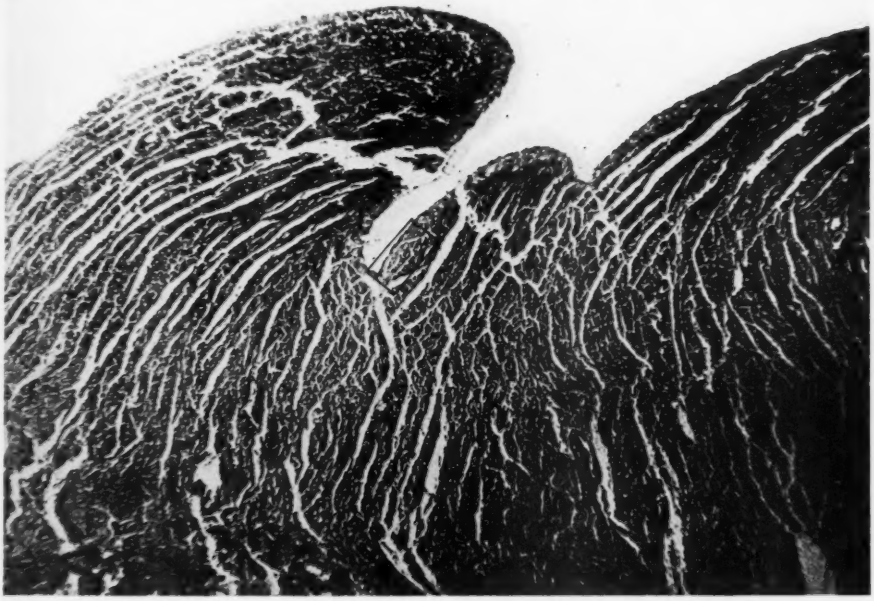
The order and orientation of the illustrations have been arranged to meet the requirements of economy and artistic reproduction. By attention to the legends the reader may avoid confusion.

PLATE 87

FIG. 1. Rabbit 36. Section through the wall of the right ventricle. Photomicrograph at low magnification. Abnormalities might easily be overlooked in this picture. The portion outlined by the rectangle is shown at higher magnification in Figure 3.

FIG. 2. Rabbit 33. Section through the aortic valve including a valve leaflet, part of the ventricular wall and part of the wall of the ascending aorta, stained by the method of Brown and Brenn.[2] Photomicrograph at low magnification. One may recognize small areas of infection on the auricular face of the valve and on the intima of the aorta. The locations of the fields represented in Figures 5 and 8 are indicated by short heavy lines near the endothelium.

1



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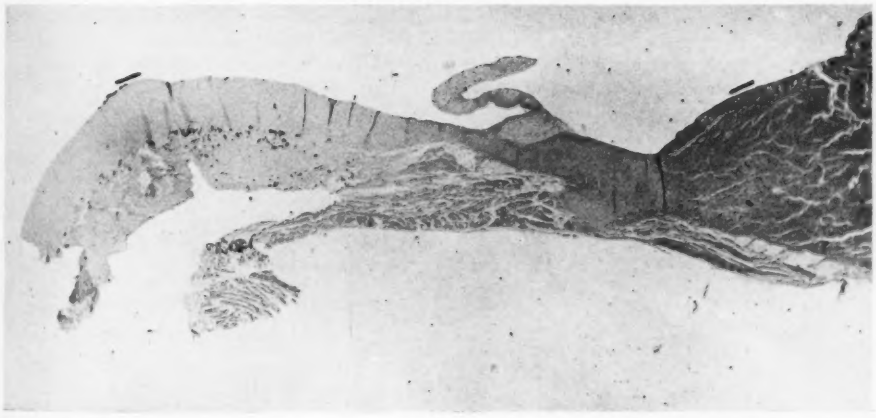


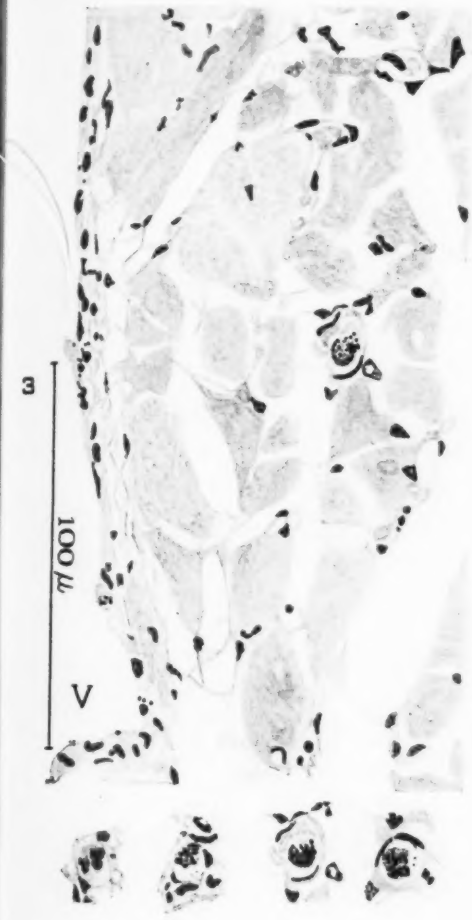
MacNeal, Spence and Slavkin Experimental Endocarditis Lenta

PLATE 88

FIG. 3. Rabbit 36. Section through part of the wall of the right ventricle stained by the method of Brown and Brenn [2] and drawn by camera lucida, objective 60 ×, N. A. 1.40, and ocular 4, at the magnification indicated by the included scale, which is standard for all colored drawings of this paper. The endocardial endothelial cells contain cocci and there are minute bits of clotted blood adherent to them in places. At about 0.1 mm. beneath the endocardium there is a thrombosed capillary containing many streptococci. Below the main drawing, this same capillary is shown in four successive serial sections of which the third in the series is the section represented in the larger drawing. It would appear that the thrombosis of the capillary has no local relation to the infection of the endocardial endothelium, which is quite general over the entire lining of the ventricle. The V is in the cavity of the right ventricle.

FIG. 4. Rabbit 34. Section through a mitral leaflet stained with hematoxylin and eosin and drawn by the same standard technic. The auricular face is roughened, and attached to it are bits of clot containing erythrocytes. The adjacent endothelial cells contain engulfed streptococci. The smooth endothelium on the ventricular face presents a sharp contrast. The V is in the cavity of the left ventricle.

FIG. 5. Rabbit 33. Drawing by camera lucida, objective 60 ×, N. A. 1.40, and ocular 4, at the standard magnification indicated by previous scales. This drawing shows a portion of the ventricular wall with streptococci in some of the endocardial endothelial cells at the upper border of the figure. A small capillary lying just beneath the endocardium is cut longitudinally for some distance and appears to be normal. The myocardial cells are fragmented.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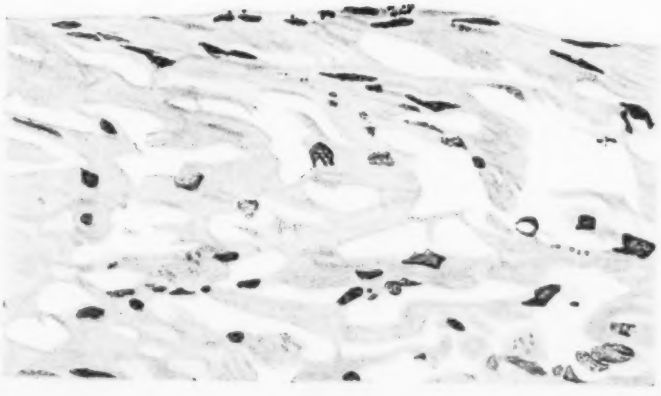

PLATE 89

FIG. 6. Rabbit 33. Drawing by the standard technic of part of the aortic leaflet in the same section as that shown in Figure 10. The endothelial cells contain cocci which are more abundant on the ventricular face (V) of the leaflet, where there is also evidence of more anatomic alteration. The location of this field is indicated in Figure 10 (V).

FIG. 7. Rabbit 34. Another section through a mitral leaflet stained by the method of Brown and Brenn [2] and drawn by the same standard technic. There are a few distinct bacteria in the endothelium on the auricular face of the leaflet and there is also an adherent clot nearly 0.1 mm. in extent, with erythrocytes and bacteria in it. The V is in the cavity of the left ventricle.

FIG. 8. Rabbit 33. Drawing by camera lucida, objective 60 ×, N. A. 1.40, and ocular 4, at the standard magnification for all colored drawings. This shows a small part of the wall of the ascending aorta. Some of the endothelial cells of the intima contain streptococci. In the upper half of the drawing the proliferation of the bacteria is associated with local necrosis.

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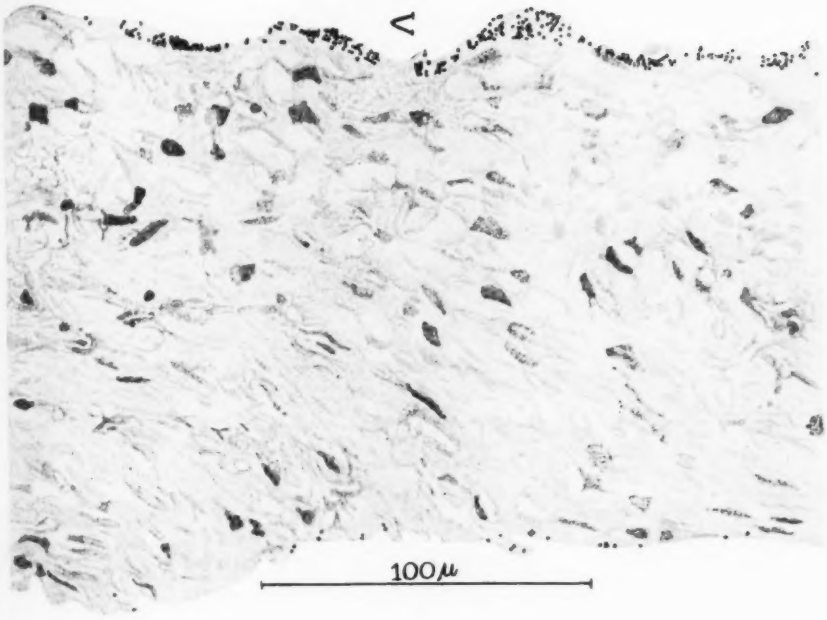

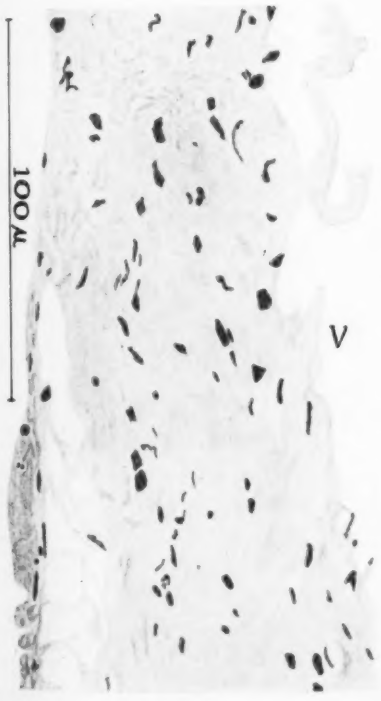

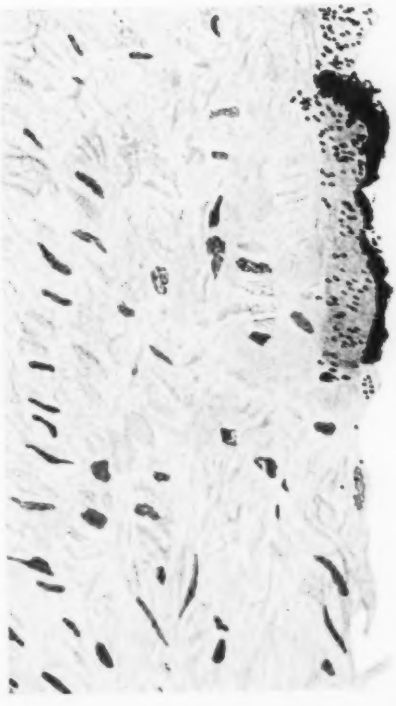

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PLATE 90

FIG. 9. Rabbit 33. Photomicrograph of a coronary artery showing at one place on the intima (lower right) a small vegetation. The artery is about 0.4 mm. in diameter.

FIG. 10. Rabbit 33. Same section as that shown in Figure 2 but photographed at a higher magnification to show more detail of the valve leaflet. The small vegetation on the ventricular face of the cusp may be recognized, as well as the collections of bacteria in the endothelium.

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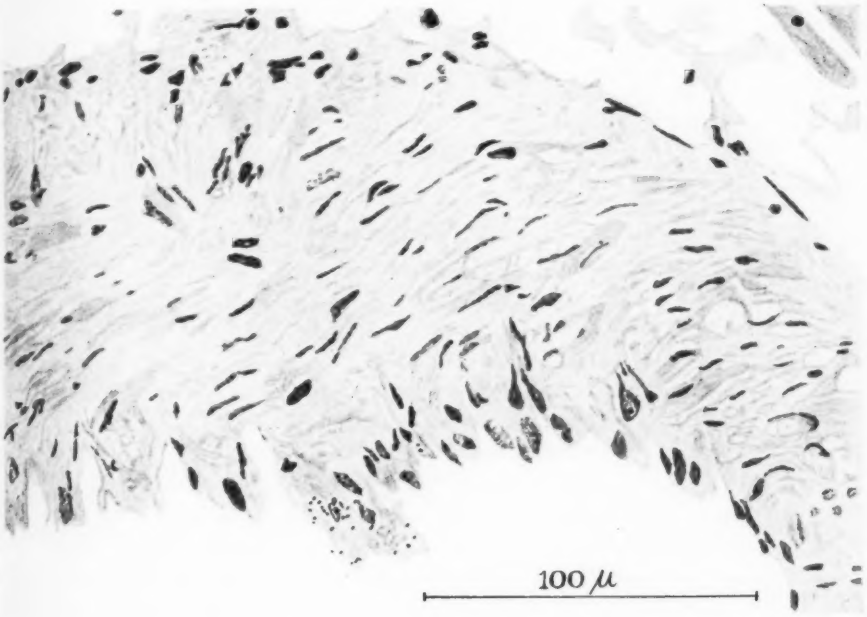
PLATE 91

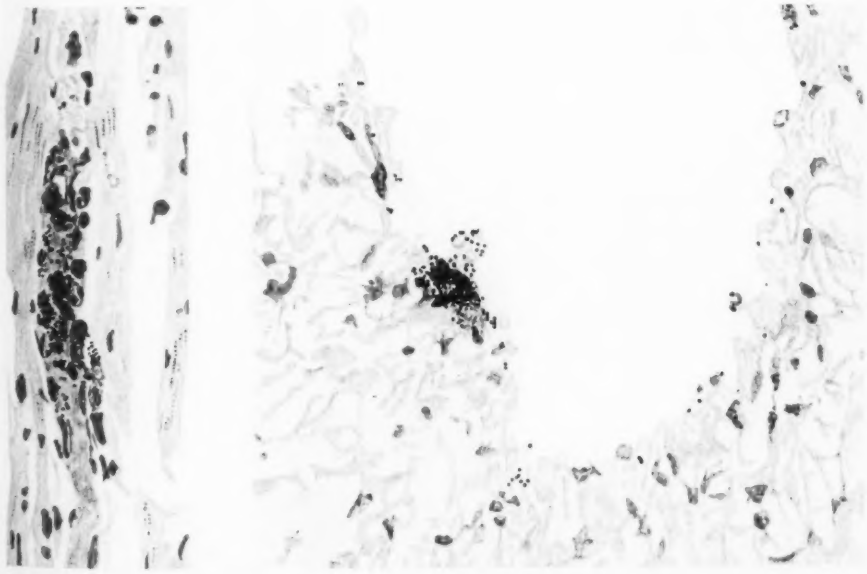
FIG. 11. Rabbit 33. Drawing by camera lucida by the standard technic. Here there is shown the small vegetation on the intima of the coronary artery seen in Figure 9. The intimal endothelium contains included cocci and clinging to it there is a deposit of fibrin in which there are erythrocytes and bacteria. The other arterial coats appear to be unaltered.

FIG. 12. Rabbit 33. Drawing by the standard technic. There is shown a thrombosed capillary in the ventricular myocardium. The thrombus is purulent, with abundant streptococci in the upper part of the longitudinal section. The lower part consists chiefly of fibrin and erythrocytes (aseptic extension of the thrombus). There is little reaction in the adjacent myocardium.

FIG. 13. Rabbit 33. Drawing by the standard technic of part of the aortic leaflet in the same section as that shown in Figure 6. Here the minute vegetation is included at the left. It has in it erythrocytes, fibrin and bacteria. At the right a polynuclear leukocyte with three included cocci adheres to an endothelial cell. The location of this field is indicated in Figure 10, in which the small vegetation serves as a landmark.

11





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Experimental Endocarditis Lenta

CHARACTERISTICS OF A LIPOSARCOMA GROWN IN VITRO *

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The recent review of adipose tissue by Wells[1] emphasized the facts that fat is a definite tissue often organoid in character and that its cells are probably not just modified fibroblasts but are derived from units which are segregated from undifferentiated mesoderm during embryonal life and persist throughout postnatal life as specialized lipoblasts.

It would seem that embryologically lipoblasts are found in two different forms: in one they are contained in tissue resembling vascular mesenchyme with widely spaced stellate or spindle-shaped cells surrounded by mucoid intercellular substance and only gradually become rounded as fine droplets of lipoid appear within the cytoplasm; in the other the cells are rounded from the beginning and grouped in gland-like lobules of a moruloid or mulberry appearance. In this form there is no intercellular mucin but the fat forms first as intracellular droplets. Both tissues are highly vascular.

It is particularly important to know about the existence of these two embryological forms when studying the malignant lipoblastic tumors because both are reproduced in the growth of liposarcomas.

The liposarcomas which reproduce the aspect of mesenchymal tissue are generally myxomatous and slimy but occasionally are composed only of fibrosarcoma-like tissue mixed with lipoblasts. Both variants usually have an admixture of adult fat cells and fat-laden phagocytes. In the second form of liposarcoma only rounded or polygonal lipoblasts of varying sizes and adult fat cells are present without any myxomatous or fibroblastic tissue. In the material at our disposal, consisting of 24 cases, there were 19 of the myxoid tumors and 2 round-cell forms. In 3 other cases the tumors were made up of solid masses of rounded cells in some areas and of myxoid tissue in others, so that they were composites of the two different forms of liposarcoma. This suggests that the lipoblast is probably a single type cell which can produce both myxoid and adenoid embryonal adipose tissue.

Liposarcomas in our experience were twice as common in males as in females and they occurred in patients with an age variation of from 28 to 82 years, with an average for the group of 56.5 years. Although

* Received for publication, January 11, 1943.

they may occur in a wide variety of regions, the great majority have developed either retroperitoneally, with a preference for the perirenal area, or in the region of the thigh, including the popliteal space, the groin and buttock. In general they tend to grow slowly to a very large size, and infiltrate surrounding tissues. Because of this they are difficult to eradicate by operation. Metastases, both in our own group and in reported cases, are known to have occurred in 20 per cent. In a small number there have been multiple tumors.

In the present communication we wish to present the cultural characteristics of a liposarcoma, since so far as we are aware such observations have not yet been recorded. The tumor developed in the subcutaneous tissues of the upper radial aspect of the right forearm of an American negro laborer, 64 years old (Fig. 1). In 8½ months it reached a size of 20 by 8 by 6 cm. and was then excised, the wound being treated with x-ray irradiation. Two courses were given during the next 5 months totaling 46 treatments through four 8 by 10 cm. fields. The factors were 200 kv.; 25 ma.; target-skin distance, 50 cm.; filters, 1 mm. Cu. and 1.25 mm. Al. The total dosage was 12,050 r.* Two years after excision a metastatic lump appeared in the subcutaneous tissue over the upper inner aspect of the right biceps brachii muscle, surrounding the cephalic vein and extending deeply between the deltoid and biceps muscles as far as the periosteum of the humerus (Fig. 1). It was excised and measured 4 by 4 by 3.5 cm. It was from this specimen that the explants were made. This area was also treated postoperatively by roentgen therapy, using three 10 by 15 cm. fields with the same factors as before and reaching a total dosage of 4,850 r.

Two months after the second operation a recurrent nodule appeared in the scar of the first operation in the forearm. This was treated with radium needles and a dosage of 1425 mg. hours was given. When the patient was last seen over 2 years later, 57 months after the first operation, there was no clinical evidence of local disease and no x-ray evidence of pulmonary metastases.

Both gross specimens were nodular, with mottled yellowish white cut surfaces glistening with sticky mucoid material. The original tumor when cut allowed the escape of a good deal of gelatinous material, brownish red fluid, and soft reddish yellow neoplastic tissue. The metastatic nodule was solid throughout.

Microscopically the original tumor showed a framework of fibrous tissue bearing blood vessels which supported an extremely loose-textured tissue containing widely spaced cells of a most diverse appearance (Fig. 3). The majority were relatively large and irregularly stellate

* Wherever "r." is used, the value indicates roentgens measured in air.

but multinucleated syncytial masses were frequent and elongated ribbon forms not uncommon. The nuclei were often hyperchromatic with prominent nucleoli and there were occasional bizarre mitoses. The cytoplasm often contained lipoid droplets. There were a few signet-ring cells. The intercellular substance had a few delicate reticulin fibers and a great many spaces which contained the mucoid material. This could not be stained with mucicarmine. There were occasional groups of adult fat cells.

The metastatic nodule from which the explants were made retained the characteristics of the original tumor but suggested much more active growth. The tumor cells were proportionally much greater in number and the intercellular substance correspondingly less. Mitosis was at the rate of one per high power field. Intracellular lipoid droplets (Fig. 3) were frequent in some areas and absent in others. Lipoid-filled signet-ring phagocytes were relatively more frequent than in the primary growth and again there was no mucicarminophilic intercellular substance (Fig. 3). The tumor was now definitely invasive and not sharply circumscribed.

Tissue Culture

Methods

Cultures were made from the metastatic lump removed from the upper arm 2 years after excision of the first tumor from the forearm. The Maximow double-coverslip, lying-drop method[2] was used, and the tissue was explanted in a medium consisting of 4 parts chicken plasma, 4 parts human placental serum, 1 part extract of adult rat spleen, and 3 parts buffered saline (according to Gey and Gey,[3] with slight modification). The cultures were washed and refed three times a week, being transferred when necessary; for this rapidly dividing tissue, passage would average once in 8 to 10 days. They were maintained for 51 days.

Growth Characteristics

The cultures grew profusely and regularly after a short lag-period of 18 to 24 hours. The outwandering of a multitude of leukocytes, monocytes and macrophages preceded growth of tissue. The tumor growth contained a few signet-ring cells of the adipose type, but these in general were inert from the standpoint of both reproduction and locomotion, and were probably drawn out passively from the explant by the contraction of the clot or by the advancing ranks of fusiform cells which formed the main body of the outgrowth. The content of the signet-ring vacuoles, however, was not fat, as ascertained by scharlach

R staining; neither could it be stained by mucicarmine nor by the metachromic dye—toluidine blue. Possibly this condition is related to the serous atrophy of fat described by Wells.[1] Often these cells progressively resolved their contents and became ameboid and spindle-shaped (Fig. 2).

The characteristic growth from the tumor (and the first tissue cells to emerge) consisted of rather fat, bright, spindle-shaped, discrete cells, with a round or oval vesicular nucleus and one or two prominent nucleoli (Fig. 5). There was considerable variation in size of both cell and nucleus. Binuclearity was common, and in the later stages of cultivation some exceedingly bizarre, lobated and fragmented nuclei made their appearance (Fig. 4).

Although the tumor was grossly slimy, we obtained no clear results in the cultures from methods designed to stain mucin. With scharlach R, however, we were able to stain many fine granules in the cells; but large vacuoles (Fig. 6) which we expected to react as fat, did not. These cells did not liquefy their plasma medium to any extent. The tumor cells did not show cement borders which blacken with silver nitrate, as do endothelial and mesothelial cells, and they never formed membranes nor the interlacing fretwork that is characteristic of fibroblastic growths. But, after about 2 weeks *in vitro,* fibroblasts and endothelium made their appearance in some cultures, especially from the necrotic edge of divided cultures. Both the branching form and the nuclear pattern (two to seven small nucleoli) of the fibroblast served to distinguish it from the lipoblast.

The cells of this tumor reacted sharply to mechanical disturbance, by contracting and remaining inert for varying periods. After transferal from one slide to another it might be as long as 3 or 4 days before cell division became frequent again. The initial "growth" of the tumor cultures appears to have been an outward migration of tumor cells, among which there were extremely few mitoses. But after this initial phase was overcome, there were as many as 75 mitoses in a culture at one time. Estimating the time required for a division to be about 1 hour, and assuming the rate of division to be constant for 24 hours, there would be 1800 mitoses during a day at the peak of activity for one of the most active cultures.

Fifteen days after the original cultures were made, a second set was prepared from the remainder of the tumor specimen, which had been kept in the refrigerator at 2° to 4°C. during that time. These cultures showed a longer lag-period—3 days or more—and produced a sparse growth, the nature of which remains undetermined.

When the original cultures were 12 days old, it was decided to use

some of them for preliminary experiments in x-ray irradiation, with the co-operation of Dr. Maurice Lenz, who was interested in the radiosensitivity of these tumors. Since we had only nine cultures whose rate of growth had been constant enough to warrant experimental use, the results can be regarded only as suggestive. These were rated from one plus to three plus on the basis of growth rate, and paired with untreated controls of parallel growth energy. Two experimental cultures, paired with two controls, were given 550 r. = 5 min., and three experimental cultures, paired with two controls, were given 1100 r. = 10 min. The factors were: 81 kv. constant potential machine, 4 ma., 25 cm. target distance, no filter, 110 r. per minute. Erythema dose = 275 r.

Irradiated cultures and controls were then kept for 1 month and compared in respect to growth (*i.e.*, number of mitoses, nutrition and cell morphology). The growth for the first 2 days was nil, among the experimental cultures. At the end of this time those which had received the smaller dosage began to divide. On the fifth day, those with the greater dosage began to divide, but the rate always lagged behind those with the light dosage, one of which was even with its paired control. Among the treated cultures, those with the highest growth rate suffered most severely. The light dosage did not affect the nutrition of the cultures significantly, but the heavy dosage produced a profound effect.

So far as morphology is concerned, there were bizarre forms appearing here and there in all of the cultures as well as in the sections of the tumor, but in the irradiated cultures the proportionate number of these forms was greatly increased. It would appear that the viable, reproductive cells were the most affected by the radiation, in some cases leaving only these stranded, bizarre cells which, from the reproductive standpoint, were already out of the running.

Discussion

In the tissue cultures of this liposarcoma the actively growing lipoblasts can be distinguished readily from common fibroblasts on grounds of nuclear as well as of cytoplasmic properties, and of general growth pattern. This is usually true, according to Hausberger,[4] of young fat cells undergoing normal development, before they have yet begun to store fat. But although a general similarity has often been noted between embryonal cells and rapidly multiplying tumor cells, the comparison should not be pushed too far, and conclusions as to normal cell origin cannot properly be drawn solely from a study of malignant cells such as these. Still it seems worth while to state that these malignant lipoblasts appear to be a distinct type of cell, and that they are rather similar to the stem cells which appear in cultures of indifferent mesen-

chyme. The typical viable, reproducing, spindle-shaped tumor cell has a number of points in common with Chlopin's [5] "desmoblast" of indifferent mesenchyme.

It may be of some value, further, to make reference to the work of Burkhardt [6] in cultivating adipose tissue from the bone marrow of adult guinea-pigs. He showed that the exaggerated capacity developed by adipose cells for fat storage is not coincident with the loss of other mesenchymal potencies. Fat cells appeared in his cultures sometimes as multinucleate giant cells which quickly degenerated, sometimes as fibroblast-like cells, more often as ameboid, wandering cells. They were capable of locomotion and phagocytosis, and even of mitotic division while containing rather large fat vacuoles. This great variability in form which they exhibited he regarded as exemplifying "the morphological richness which characterizes vascular mesenchyme" from which he believes adipose tissue to be an offshoot.

It is interesting that in both sections and cultures of the liposarcoma which we have described, the gamut of cells discussed by Burkhardt [6] is run. It is our observation also that the bizarre, multinucleate cells are relatively nonviable, and are on the verge of degeneration. Lipoblasts distended with vacuoles may resolve these partially and become ameboid (Fig. 2). The tumor cells may also develop the cytoplasmic (though not the nuclear) aspects of fibroblasts, while containing large vacuoles (Fig. 6). In our cultures these large vacuoles have not contained fat, but may have illustrated the capacity, referred to by Wells,[1] of depleted fat cells to take up water.

Summary

The two histological types of liposarcoma are discussed briefly, and the clinical and histological aspects of one case are recounted.

Portions of a metastatic nodule from this case have been cultivated *in vitro*, and the growth characteristics are described. These malignant lipoblasts appear to be a distinct type of cell, though highly variable in form.

Some of the tissue cultures of this tumor have been exposed to x-ray irradiation, and their subsequent course is summarized.

The authors' thanks are due to Dr. Cloyce F. Bradley, who assisted in the preparation of the tissue cultures.

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[*Illustrations follow*]

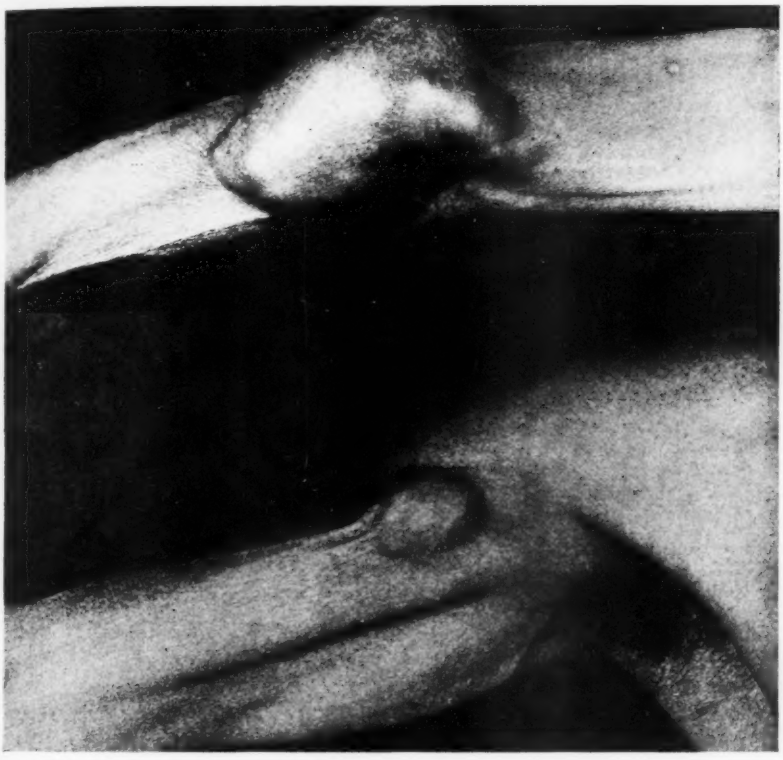
DESCRIPTION OF PLATES

PLATE 92

FIG. 1. Liposarcoma of the forearm. Above is the primary tumor and below the metastasis in the upper arm.

FIG. 2. Lipoblasts at the periphery of outgrowth from 4-day culture. There are signet-ring cells in various stages of elongation and vacuole resorption: 1, 2, 3, 4, 5. Formaldehyde fixation, toluidine blue stain. × 680.

1



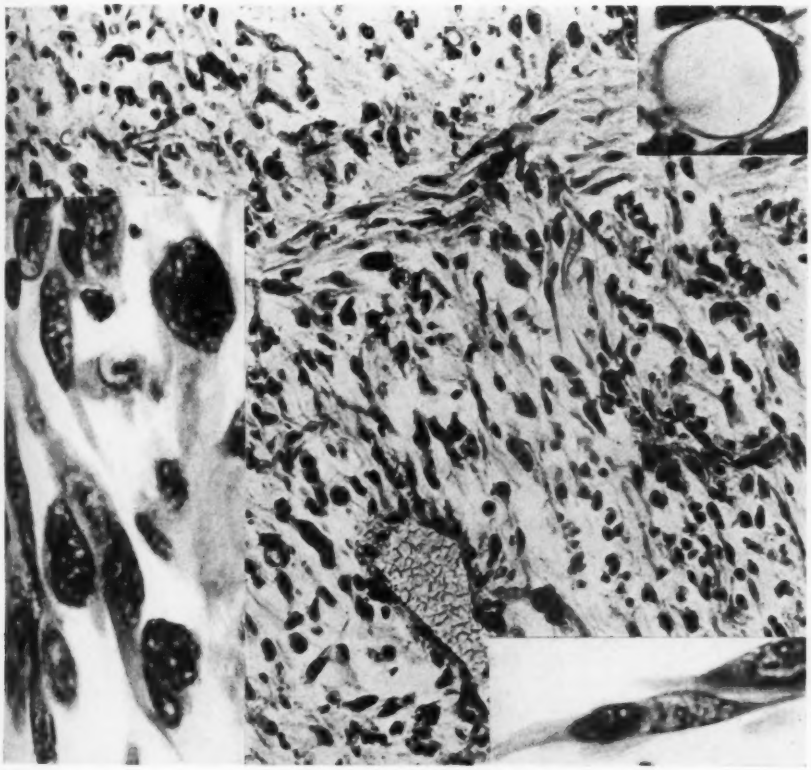
2

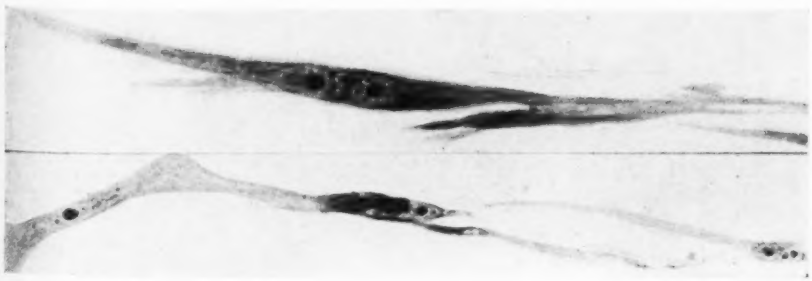
Murray and Stout Liposarcoma Grown *in vitro*

PLATE 93

FIG. 3. The low-power photomicrograph shows the characteristics of the original tumor in the arm. The insets show cells of the metastasis from which cultures were made. Bizarre elongated cells (lower left); signet-ring cell (upper right); lipoblast with vacuoles (lower right). × 630.

FIG. 4. Tumor cells from 42-day culture, irradiated 550 r. = 5 min. Note disparity in cell size, vacuoles, and lobated nucleus in large cell. Helly's fixative, mucicarmine stain. × 380.



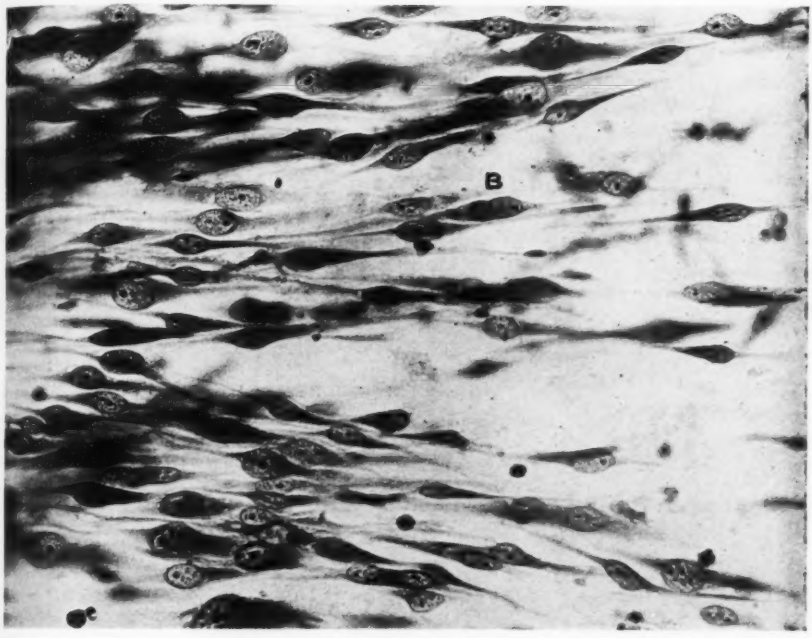


Murray and Stout Liposarcoma Grown *in vitro*

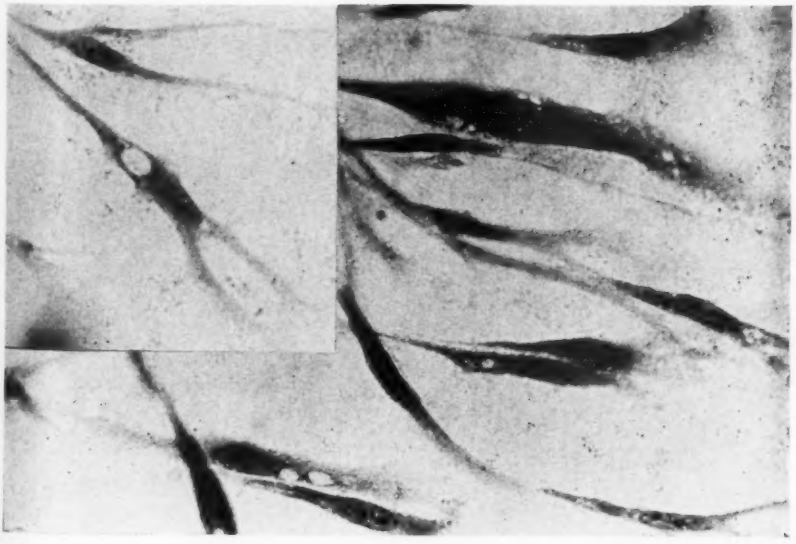
PLATE 94

FIG. 5. Typical outgrowth of viable, reproductive cells after 7 days *in vitro*. Note spindle shape, 1 or 2 nucleoli, and lack of uniformity in size among the cells. B = binucleate cell. Zenker's fixative, fuchsin-ponceau-aniline-blue stain. × 380.

FIG. 6. Large watery vacuoles in branching tumor cells of 42-day experimental culture (550 r. = 5 min.). Helly's fixative, mucicarmine stain. × 380.



5



6

Murray and Stout Liposarcoma Grown *in vitro*

EPITHELIAL CYSTS AND CYSTIC TUMORS OF THE SKIN *

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Cysts of the skin form a heterogeneous group which is difficult to subdivide on either an etiologic or histologic basis, as a cyst often carries no earmark indicating its origin. A reinterpretation of the abundant literature on cutaneous cysts would be impractical and of doubtful value as well, because so much of it is vague and uncritical. This review is based largely on our observations of 566 epithelial cysts of the skin examined in this laboratory over a 20-year period. Recent reports of large numbers of cysts by Caylor,[3] who reported on 236 "cutaneous cysts," Bishop,[1] reporting 119 "sebaceous cysts," and Stone and Abbey,[10] also reporting on 363 "sebaceous cysts," have been especially helpful.

Cysts of the skin may be put into one or another of three main groups according to their origin: (a) aberrant squamous epithelium, either congenital or traumatic in origin; (b) overactivity of glands, resulting from general physiologic forces, external conditions, or from some unknown factor; (c) degenerative changes of skin appendages or of benign or malignant epithelial tumors.

A. CYSTS

1. Epidermal Cysts

The commonest type of epithelial cyst of the skin is the epidermal cyst which is lined entirely by stratified squamous epithelium usually without skin appendages. All but 10 of our 566 cysts fall in this group. These cysts may arise from hair follicles, sebaceous ducts and from the epidermis, and it is usually impossible to determine the parent structure. Many different terms have been applied to them, generally depending on the probable structure of origin, as: sebaceous, retention, follicular cysts, atheroma, pseudo-atheroma, steatoma, milia, sebo-epidermal cysts, sebaceous tumors, oil cysts, squamous epithelial cysts, pilosebaceous cysts, sebaceous cysto-adenomas, keratomas, simple dermoids, and epidermal inclusions.[2, 5]

The cysts which are clearly derived from one or another of the cutaneous organs will be discussed separately.

The simple epidermal cyst lies in the corium, rarely in the subcutaneous tissue. Those cysts occurring along embryonic lines of

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closure and situated deep in the corium have been assumed to be congenital inclusions. Some of the cysts are joined to the surface by epidermal cords, suggesting that for some reason abnormal downward prolongation of the epidermis had resulted in a cyst. But the majority of these cysts are not connected with any cutaneous structure. Injury to the skin may produce epidermal cysts by misplacement of epithelium or stimulation of epithelial proliferation.

These cysts contain compact, fine flakes of epithelial cells. The contents may disintegrate into amorphous débris with fat and cholesterol or they may become hard, keratinized tumors and often calcify. A similar material found in sebaceous cysts is more fatty and is distinguished by the characteristic rancid odor. Internal papillae and ridges have been described as features of cysts formed from epidermal inclusions.[12, 13] Absence of any connection with the skin surface distinguishes them from follicular or sebaceous cysts.

The majority of cysts that are removed show rupture of the capsule with inflammatory reaction. A foreign body giant cell reaction and mononuclear cell infiltration are elicited by the contents of the cyst which are forced into the fibrous tissue of the corium. This reaction may resemble a lipophagic granuloma[6, 7] (Fig. 1). The portions of cyst wall which are isolated often proliferate actively and may simulate a malignant, infiltrating growth. A few cysts remain stationary; others undergo spontaneous involution, with fibrosis, calcification, and occasionally ossification. Eleven of our 566 epidermal cysts were calcified; and one cyst was ossified. Malignant change is probably extremely rare.[4, 8, 9-11] We have encountered carcinomas arising from the walls of four epidermal cysts (Fig. 2).

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2. Epidermal Cysts of Traumatic Origin

Trauma has long been considered an important factor in the formation of epidermal cysts [1] which have been variously diagnosed as traumatic, post-traumatic,[13] implantation epidermal or implantation dermoid cysts.[5] This assumption has been corroborated experimentally. Kaufmann [12] found that cocks' combs buried in subcutaneous tissue formed cysts lined by stratified squamous epithelium. It has also been thought (erroneously) that cysts may develop from epithelization around foreign material such as a magnesium disk or camphor oil after it has been absorbed.[15, 17]

Trauma may produce cysts in three ways: (a) by displacement of epithelium; (b) by altering the structure of cutaneous organs, such as hair and glands; (c) by stimulation of epithelial growth. Cysts sometimes follow continued irritation, through stimulation of growth of epithelial structures in their natural location.[19] Kummer and Christiani [14] reported epithelization and cyst formation at the end of an embedded nail. This epithelization is said to derive from epithelium of skin appendages, particularly the ducts of coil glands.[13] Franke [9] suggested that embryonic rests pinched off from the epithelial pegs might become cystic as a result of trauma. Although trauma plus irritation of normal cutaneous epithelial structures or of dormant embryonal rests may be the primary cause of some cysts of the skin, the evidence is not always enough to establish the cause. Such a diagnosis has often been made on insufficient grounds.[7]

Implantation cysts form one variety of the traumatic cysts. In this group may be included the cysts that develop in postoperative scars,[2, 20, 26, 28] and probably some of the cysts said to be common on the fingers of laborers.[10] Tooker [23] reported a post-traumatic, presumably implantation cyst in the posterior chamber of the eye and Couch [4] reported one beneath the periosteum of the skull. Wien and Caro [25] suggested that subcutaneous implantation of epithelium would be more apt to occur from injury with a blunt or tearing instrument.

But Unna [24] cited a case of epidermal cyst which was said to result from a needle prick, and he expressed the opinion that the implant is more apt to survive if spherical, since irregular implants stimulate granulation tissue and may be absorbed.

In most instances no distinction can be made between cysts resulting from implantation of epithelium and those from epithelial proliferation *in situ*, and in any case such a distinction has no practical importance.

There is no very clear information as to the incidence of traumatic epidermal cysts, although several single cases as well as groups of cases have been reported.[3, 13, 16, 18, 22, 25] Wörz [27] reported a series of 55 cases in which 44 per cent of the cysts were said to be related to injury.

The latent period may be anywhere from 25 days to 25 years.[18] The rate of growth is also variable and both infection and calcification occur. Cogswell and Goodale [3] reported one cyst that eroded the terminal phalanx of the finger. Franke [8] reported malignant proliferation in what may have been a true traumatic epidermal cyst of the base of the thumb. Some epidermoid carcinomas developing after injury have been attributed to malignant change of misplaced epithelium, but without actual proof.[6, 11] Certain adamantinomas of the tibia have been said to originate the same way.[21]

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3. *Sebaceous Cysts*

The term sebaceous cyst has been made so inclusive that it has lost its essential meaning. Virchow [16] considered all epithelium-lined cysts of the skin containing semisolid or fluid contents to be formed from sebaceous glands. This concept has been generally accepted with very little critical consideration. Several rather loose terms have accumulated for the main class of sebaceous cysts or certain histologic variations thereof: [1] "calcinoses," [17] "sebo-epidermal cysts," [11] "steatoma," [12] "atheroma." [10] Stelwagon and Gaskill [12] derived sebaceous cysts from embryonic misplacements. Franke [8] and Török [14] were the first to differentiate epidermal cysts, "atheromas," and sebaceous cysts; but others, such as Sulzberger and Wolf,[13] while recognizing the distinction, did not apply it clearly.

The tendency to inflammation and degeneration shown by all types of cysts makes it frequently impossible to tell one from the other.

Unna [15] thought that cysts of the sebaceous gland proper had not been shown to exist, and that so-called "sebaceous cysts" are really epidermal cysts and may be formed by a downward keratinization of the pilosebaceous follicle and later distention to form cysts. Broders and Wilson [3] also concluded that most sebaceous cysts are "keratomas," that is, epidermal cysts filled with laminated keratin.

The frequency of malignant change in sebaceous cysts has been placed as high as 9.2 per cent,[2] but Caylor's [4] more conservative figure, 3.4 per cent, is more in accord with the general impression.[7, 9]

We have accepted the definition, long in use, of sebaceous cysts as retention cysts of sebaceous glands.[6] Secreting sebaceous gland cells must form an integral part of the lining. We have identified only 3 such cysts among 566 cutaneous epithelial cysts, comparable to Collins' [5] experience (Fig. 2). A helpful gross diagnostic point is the homogeneous, yellow, buttery consistency of the contents of sebaceous cysts as contrasted with the waxy, flaky, white material characteristic of epidermal cysts. Infection gives a darker color and a rancid odor to the contents of sebaceous cysts.

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4. Cystic Dilatation of Sweat Glands

The only true cyst of the sweat gland involves the deep part of the duct and is usually called hydrocystoma.[9] Other cysts are less clearly neoplastic, as, for example, the transient cystic dilatation of the terminal duct seen in sudamina [8] or prickly heat, and the dilatation of coils which occurs in a variety of conditions.

Cystic dilatation of sweat glands was described first as hypertrophy by Verneuil,[12] later as dysidrosis by Jackson,[4] and then as hydrocystoma.[1, 3, 5, 6, 9] Little has been added to the literature since these early reports. There have been a few recent reports.[2, 7, 13] We distinguish this lesion from syringoma.

The cysts present as pinhead to pea-sized, shiny, translucent, somewhat deep and persistent vesicles on the face, especially the forehead, eyelids, and cheeks. Women in late middle life are said to be most often affected. A peripheral hyperemia is sometimes present about the large cysts. If unmolested, they may in time disappear as a result of excretion or absorption of the fluid, but occasionally they persist unchanged.

Relatively little is known of their natural history. They have been considered an anatomicophysiologic aberration, rather than a neoplasm.[10] An excessive secretory activity is more or less borne out by the histologic structure and the multiplicity of lesions. In certain instances they seem to be associated with exposure to heat and moisture, either climatic or occupational. The importance of nervous influences is in great part hypothetical.[3, 6]

The cysts may be large enough to cause some distortion of the adjacent corium but this is not usual. The lining is the flattened distorted epithelium of ducts, which apparently keratinizes in some instances.[2, 7] Sutton and Sutton [11] described a sensitivity to radiation.

The only cyst of sweat gland which we have in our group of skin tumors had been excised intact and measured 0.8 cm. It was filled with glairy fluid and the inner surface was white, smooth and glistening. The epithelial lining, though partly exfoliated, was in other parts well preserved and consisted of tall columnar cells, the nucleus at the base and the clear cytoplasm toward the lumen, and having a basal layer of

flattened cells. Other partially cystic sweat glands were adjacent to this larger cyst. In the surrounding corium there was a slight inflammatory reaction, edema and hemorrhage. The overlying epidermis was unchanged except for being somewhat thinned.

While cysts of Moll's glands have been described, none was encountered by us.

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5. *Dermoid Cysts*

True cutaneous dermoid cysts are of necessity congenital and occur in relation to embryonal fissures and clefts. For their diagnosis the occurrence of epidermal epithelium and all skin appendages is requisite. In addition to these, various tissues of mesenchymal origin may be present. Dermoids of the skin are restricted to the corium and must be kept distinct from branchial cysts. At times their differentiation from traumatic epithelial cysts or from teratomas may be difficult unless the above criteria are followed. Whether there is any essential difference other than location between dermoid cysts of the skin and some of the simpler teratomas is questionable.

The contents of the cutaneous dermoid resemble the material within the more familiar ovarian dermoid cysts. Hairs are embedded in a pale yellow, greasy, pultaceous mass, which often has a faintly rancid odor. Usually these cysts do not reach a size over a few centimeters in diam-

eter, and growth is slow or negligible. Sometimes one or another element may be atrophic, hyperplastic, or rarely show malignant change.[2] Gans [3] mentioned that calcification of the contents and subsequent ossification may occur.

True dermoids of the skin are rare.[1, 4] The majority of those reported near the skin surface are either from branchial clefts or from the lines of closure of the nasal bones or skull.[6] One, containing teeth, was reported as occurring in the external auditory canal.[5]

In our material we have encountered only two cutaneous dermoid cysts; each arising above the outer canthus (Fig. 3).

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6. *Multiple Follicular Cysts*

Cysts arising from the pilosebaceous apparatus were first reported by Bosellini [2] and Pringle.[18]

Abnormally active keratinization is usually considered the primary change which may give rise to cysts in the following manner: (1) gradual distention of a follicle from accumulation of detritus of dead keratinized cells; (2) blocking of the sebaceous duct; (3) accumulation of sebaceous secretion.[13, 14] The sebaceous gland probably plays only an accidental or passive part, although it may add to the contents or make up part of the wall. In the older cysts, the sebaceous gland is often atrophic, and sometimes it is absent. It is obvious that the distinction from a sebaceous cyst might not always be possible, and there may be confusion with dermoid cysts.[16]

The cause of the hyperkeratinization is not known. Weidman [22] has frequently found these cysts on tuberculous patients at autopsy and has suggested that avitaminosis may be a factor in their development. There is a general belief that the condition is hereditary.[7, 17] Klausner [10] and Sachs [20] reported the lesion in three successive generations, and Sachs reported one family in which ten members were affected. Weber [21] believed that the condition occurs in two forms: one which is

mild and localized and not hereditary, and the other, which is more generalized and severe and a mendelian dominant characteristic.[5]

These cysts have certain clinical characteristics which seem to separate them from the main group of epidermal cysts. Multiplicity is the rule, several hundred sometimes being present,[8] but solitary lesions have been described.[6, 15] They are most common where the pilosebaceous apparatus is well developed and first appear on trunk, scrotum and axilla. Many of the patients have a long history of seborrhea or acne.[9, 11, 12] The cysts are often present at an early age but may attract attention first in middle life.[3, 4, 19]

The gross aspects are not peculiar. The individual lesions are covered by smooth skin which is more or less fixed to the underlying nodule. The majority of these cysts are 0.8 to 1.5 cm. in diameter, although some very large cysts have been reported, as that of Chiari[4] which measured 6 by 13 cm. They may be slightly blue or yellow. The contained material, made up of dead, exfoliated, keratinized cells, has the same flaky consistency as the material in epidermal cysts, but the addition of sebaceous secretion sometimes adds a waxiness or milkiness, and some cysts are described as containing an oily fluid.[1] Like epidermal cysts, they are subject to infection and are often obliterated by inflammation or fibrosis. Malignant change probably does not take place.

We have four follicular cysts in our material.

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B. Cystic Benign Tumors of Familial Nature

"Epithelioma Adenoides Cysticum"

Cystic epithelial tumors of the skin are very common. They may be divided into two main groups: those which are multiple, with certain clinical and histologic characteristics suggesting a congenital origin; [34] and those which are primarily tumors of middle age and may be classed with the nonkeratinizing tumors. There is no sharp dividing line between the two groups either on a clinical or a histologic basis. Nonkeratinizing epithelial tumors of the skin, of which the basal cell carcinoma is the frequent form, are prone to cystic degeneration of both the tumor and the stroma.[21] Such tumors have often been confused with the true cystic tumors of sweat glands. It is too seldom recognized that tumors of entirely diverse histologic structure may present identical clinical features. For example, the common epithelial tumors of the skin, epidermoid carcinoma and basal cell carcinoma, are not always distinguishable clinically even by experienced observers. In this paper we have to deal with a similar problem, *viz.*, two groups of skin appendage tumors that are often confused clinically, but are histologically distinct.

The clinical condition under discussion, relatively rare, is characterized by multiple small tumors, averaging 0.5 cm. in diameter, usually scattered either over the chest and axillae or on the central part of the face and lower eyelids, but sometimes in both locations.[40] The gross lesions are discrete domed nodules, colorless, slightly discolored, or distinctly yellow, covered by smooth intact epidermis. This syndrome is usually, but not always, familial and occurs more often in females, especially at puberty.

Those who believe the process can be subdivided clinically state that the tumors occurring on the face are epithelioma adenoides cysticum, and those occurring primarily on the chest are syringoma, although the gross appearance of the lesions is similar, regardless of locality.

Only a very few microscopic examinations have been possible in proportion to the number of lesions on a given person. As a result, we know relatively little about their structure. There appear to be at least two histologically distinct tumors that may have these clinical and gross features. One resembles the duct of a sweat gland and the other a tumor of hair follicle.

The tumor of sweat duct type is said to be located primarily on the chest. It is less apt to be familial and is said to be radiosensitive. The tumor resembling hair follicles, on the other hand, is described as a distinctly yellow tumor [45] primarily on the center of the face and lower eyelids, that is radioresistant,[10] and is familial with a few exceptions.[3, 13, 26, 43] The tumors have been described in several members of a single generation and in successive generations.[7, 13, 31, 39, 41]

The duct type of tumor [19, 42] (syringoma) was discussed with tumors of sweat glands. We will describe briefly the tumors of hair follicles as seen in this condition. The microscopic structure is essentially that of a tumor of hair follicle,[20, 23, 27, 32, 39, 44, 46] in which there is cystic degeneration and also some proliferation of the matrix. The follicle and the matrix cells may retain their specific characteristics, with a typically benign structure, although there may be transitions to hair matrix carcinoma. The tumors have been ascribed to the fully differentiated hair follicle,[20] lanugo hair,[6] or undifferentiated anlagen [23] which are stimulated to growth at puberty.[22] Such a sequestration of epithelium seems most likely to occur along body fissures [29] but many tumors cannot be accounted for in this way.

Unfortunately this tumor has never been given a definitive name. The least controversial of the terms by which it has been known is Brooke-Fordyce disease. Although it is now most often called epithelioma adenoides cysticum, there are two objections to this term. It is too vague: all nonkeratinizing epitheliomas tend to become cystic. And it has been used for a loosely defined clinical entity which may result from enlargement of sebaceous glands [36] as well as tumors of sweat glands and of hair follicles or basal epithelium.[9, 16] It does not connote a single clearly defined pathologic process, and has been used for quite unrelated conditions.[8, 38] Nevertheless, epithelioma adenoides cysticum as a descriptive term for multiple benign cystic lesions of hair-follicular origin is sufficiently well established to use so long as it is assigned to this specific lesion.

By no means has there been general accord as to the histologic structure or proper interpretation of the syndrome of multiple benign epithelial tumors.[18] This is well illustrated by the differences of opinion on some of the early cases.[2, 4, 12, 37, 45] The histologic structures of basal cell carcinomas, hair matrix carcinomas, and sebaceous adenomas have all been described in this syndrome of benign familial tumors of the face and chest.[12, 15, 24, 25, 30, 46] Other congenital tumors such as the so-called cylindroma of the scalp may also be present.[5] It is well to remember that benign skin tumors, as nevi, neurofibromas, lipomas, tumors of sweat glands, tend to be multiple; and also that multiple epithelial cutaneous tumors usually have an organoid structure and are for the most part nonkeratinized and benign. Considering the embryologic relationship of the epidermis and cutaneous appendages, a congenital dystrophy of dermal structures might well yield a variety of unusual epithelial structures. In only rare instances have any of these tumors been reported as clinically malignant.[1]

The following terms have been applied to the general group of benign familial tumors which we have described in this paper:

1. Benign epitheliomata with colloid degeneration.[35]
2. Epithelioma adenoides cysticum.[6] (This is the term most widely accepted.)
3. Multiple benign cystic epithelioma.[12]
4. Tricho-epithelioma papillosum multiplex.[20]
5. Acanthoma adenoides cysticum.[45]
6. Hydradenome éruptif.[19]
7. Multiple cystic symmetric nevi.[47]
8. Tricho-epithelioma papillosum with syringocystadenoma.[28]
9. Cystic basocellular epithelioma.[17]
10. Naevus follicularis.[39]
11. Brooke's tumor.[14]
12. Adenoid cystic epithelioma.[11]

Treatment has not been successful since the multiplicity of lesions usually eliminates surgery and the tumors do not react uniformly to irradiation.[10, 30] It has been said that growth may be retarded by radiant energy and the partly degenerated cystic tumor expressed or excised.[39] However, the resulting scar may be just as objectionable as the tumor. If there are only a few tumors, either surgical excision or carbon dioxide snow or electrodesiccation may be satisfactory.

Malignancy has been described following irritation or improper treatment. Among the 116 cases in the literature which were reported in detail, there were 5 cases of secondarily arising carcinomas, an inci-

dence of 4.3 per cent. The carcinomas are usually of basal cell type,[24] but Gordon [14] reported an intra-epidermal epithelioma.

Summary. We use the term epithelioma adenoides cysticum for a benign lesion of hair follicles which is grossly indistinguishable from syringoma and, like it, multiple and familial. The so-called syringomatous type is properly classified with tumors of sweat glands.

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C. Calcified Cysts and Calcified Epitheliomas

The number of reports which have appeared on "calcified epithelioma" seem out of proportion to its clinical significance. As Ewing [4] has pointed out, minor focal calcification occurs frequently in all kinds of epithelial tumors and probably represents a regressive process usually associated with sluggish growth and necrosis. A marked degree of calcification is less common and complete calcification of a tumor rare. Tanasescu and Balan [19] have reported a large series of calcified cutaneous epitheliomas.

We interpret most of the descriptions and photomicrographs of the reported calcified epitheliomas as compatible with calcification of the contents of an epidermal cyst.[2, 7, 9] Nevertheless, the true calcified epithelioma has been generally recognized and carefully described in the literature. "Calcified epithelioma" was early made familiar by Malherbe,[14] who described calcified tumors of sebaceous glands and later noted a similar calcification of cysts of sweat glands.[15] Unna [20] pointed out that tumors of the scalp and face are especially apt to calcify. The following list shows the different types of tumors which have been considered important in the formation of calcified epitheliomas:

1. Nevi (*i.e.*, congenital tumors) [5, 6, 8, 10, 11]
2. Sebaceous gland tumors, either from anlagen or differentiated glands [13, 14, 18]
3. Epidermal cysts [7]
4. Epitheliomas arising from epidermal cysts [7]
5. Calcified carcinomas [1, 3, 7, 12, 21]
6. Calcified basal cell tumor [2]

Ossification of calcified tumors may occur [17] and Musger [16] described a case of basal cell carcinoma in which osteogenic sarcoma arose.

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[*Illustrations follow*]

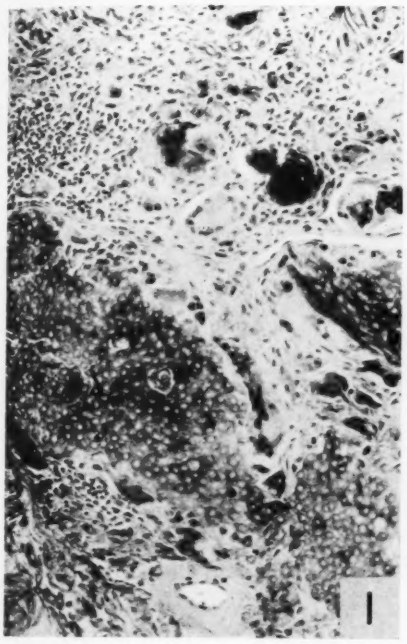
DESCRIPTION OF PLATE

PLATE 95

FIG. 1. Epidermal inclusion cyst with chronic inflammation and foreign body giant cell reaction. Phosphotungstic acid hematoxylin stain. × 150.

FIG. 2. Sebaceous cyst with sebaceous gland carcinoma developing from one margin. Hematoxylin and eosin stain. × 12.

FIG. 3. Dermoid cyst of skin. Multiple hairs are present in the cyst. Phosphotungstic acid hematoxylin stain. × 19.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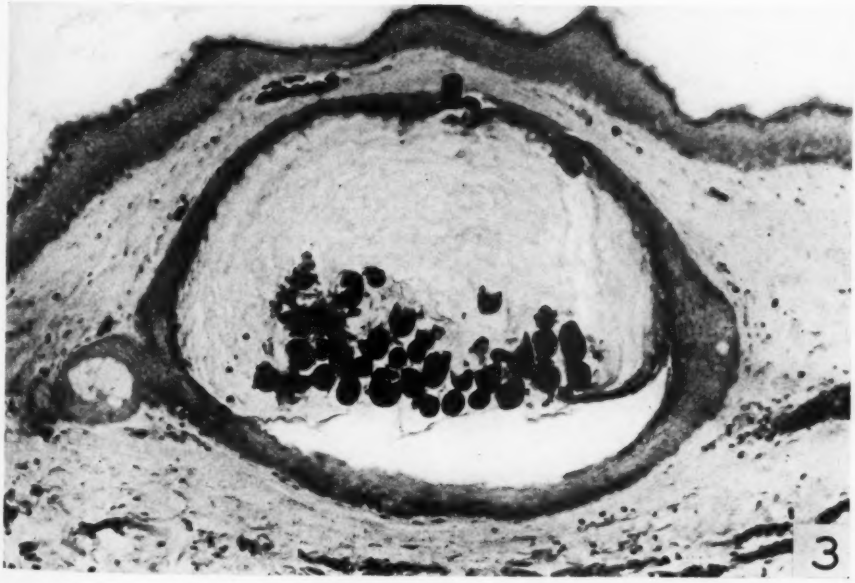
3

DIETARY ULCERS OF THE ESOPHAGUS OF THE RAT*

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In the course of determining the long-term effects of a deficient rice diet on white rats, peculiar lesions of the esophagus were noted in a number of animals. The most striking and apparently the farthest advanced alterations were focal ulcerations of the mucosa accompanied by various stages of penetrating inflammation. Other changes included mucosal hyperkeratosis and edema of the submucosa, atrophy and fibrosis of the muscularis, and dilatation of the organ itself, at times without demonstrable cause. Essentially similar lesions of the forestomach of the rat were described by Pappenheimer and Larimore [1] in 1924. These authors implicated a mechanical as well as a dietary factor. Since the morphology of the lesions in their group as well as in mine suggested the participation of a mechanical factor, it was necessary for me to exclude this in order to establish a dietary etiology for the rat esophageal ulcers. The production of the esophageal lesions and their morphology in *Group I,* and their prevention by a largely synthetic vitamin diet in *Group II,* are described in this paper.

METHODS AND RESULTS

Group I

Seventeen white rats of the Osborne-Mendel strain, between 2 and 3 months of age, were placed on a diet of fresh, finely ground, unpolished Texas rice mixed with cottonseed oil in the ratio of 20 cc. of oil to 1000 gm. of rice. This diet was supplemented with a small slice of fresh carrot, each rat receiving daily approximately 1 gm. The rats were fed the diet and given water *ad libitum.* (See Sugiura and Rhoads [2] for a discussion of this diet.)

Only one rat died a significant time before the experimental period of 6 months was over. Three died a week previous to this time. All of the animals gained some weight during the experimental period, but many of them lost weight after the first few months. At the end of the experimental period two weighed less than their original weight (Table I). Two of the rats (39L16 and 31L61) were fed the diet for 8 months before being killed. One of these had multiple typical esophageal ulcerations, the other showed mucosal thickening and questionable hyperkeratosis.

* Received for publication, December 10, 1942.

TABLE I

Experimental Data for Group I

Rat no.	Esophagus	Born	Diet began	Killed	Beginning weight	Maximum weight	Dead weight
					(*gm.*)	(*gm.*)	(*gm.*)
39L16	++	9-2-41	11-12-41	7-7-42	140	180	145
52L20	++	9-10-41	11-12-41	5-31-42	145	180	154
40L16	++	9-2-41	11-12-41	5-24-42*	135	185	122
23L63	++	7-22-42	10-9-41	3-25-42	210	280	210
17L63	++	7-25-42	10-9-41	3-25-42	157	210	180
19L51	++	7-14-42	10-9-41	3-19-42*	164	255	255
24L60	++	8-23-42	10-21-42	3-25-42	133	205	210
41L16	++†	9-2-41	11-12-41	5-26-42	160	200	145
32L61	+	8-29-41	11-12-41	5-24-42*	130	190	122
29L61	+	9-29-41	11-12-41	4-11-42*	180	240	180
38L16	+	9-2-41	11-12-41	5-31-42	120	130	100
14L51	+	7-14-42	10-9-42	3-25-42	130	175	160
3L3	±	7-26-42	10-9-42	3-25-42	148	195	180
31L61	±	8-29-41	11-12-41	7-7-42	160	210	205
16L22	o	7-22-42	10-9-42	3-25-42	213	285	260
4L3	o	7-26-42	10-9-42	3-25-42	148	205	195
5L3	o	7-26-42	10-9-42	3-25-42	140	190	150

++ Ulceration and hyperkeratosis.

+ Hyperkeratosis. 38L16 showed mucosal erosion; 32L61 showed dilatation of esophagus in addition to hyperkeratosis.

* Died.

† Ulceration located in forestomach instead of in esophagus.

Some of the rats had a moist, reddish brown discoloration around the nose, and many developed a coarse, shaggy coat. A few of the animals lost hair on the abdomen and in the groin and axilla. The conspicuous lesions of the ears and paws described by Antopol and Unna [3] as characterizing B_6 and pantothenic acid deficiency were not encountered. Neither was their general appearance suggestive of vitamin A deficiency. Eye signs, as well as infections in the cervical region or urinary tract, were absent except for some blepharitis in one animal. The rats increased in size but most of them appeared to be malnourished at the end of the experimental period.

No consistent abnormality was noted in any organs other than the esophagus. The esophageal lesions, when fully advanced, were quite striking (Figs. 1 to 5). Seven of the 17 rats had ulcers in the lower esophagus and 1 had an ulcer of the forestomach. The ulcers were occasionally confluent and in 1 rat extended above the mid-esophagus. The larger ones were of the penetrating type, as attested by contiguous inflammation of the liver, lungs and pleural cavity. The mucosa about the ulcers was wrinkled with white islets and ridges of leukoplakia. Small ulcers were noted in these leukoplakial spots, and the edges of the large ulcers were raised, white and glistening (Fig. 3). The esophagus was dilated to varying degrees about the ulcers and above them. Masses of rice were frequently found impacted in these sac-

cular dilatations. Digital palpation of the rice disclosed a distinct sharpness of the granules.

In rat 32L61 no ulcers accompanied the rather extensive dilatation of the lower and mid-esophagus. In rat 40L16 advanced ulceration was present in addition to the sacculation of the organ (Fig. 1).

All of the esophagi were sectioned, and from most animals sections of the lung, forestomach, renal pelvis, liver and bladder were taken. The large esophageal ulcers were essentially similar. The squamous epithelium at their edges was thickened in all layers and usually was hyperkeratinized (Fig. 12). Numerous mitoses were found in the basal layers of the adjacent squamous epithelium. The granulation tissue at the ulcer bases was rich in capillaries and was crowded with eosinophils and polymorphonuclears and contained some lymphocytes, plasma cells and macrophages. Fibroblastic activity was advanced and edema of the submucosa was conspicuous. In many ulcers, fused eosinophilic collagen and disintegrating muscle fibers formed the base, an appearance not unlike that seen in peptic ulcers and sometimes attributed to contact with gastric juice. Masses of bacteria were seen in the large necrotic ulcer craters. In one rat there were sizable varicosities of the deep esophageal veins beneath a zone of mucosal erosion and ulceration.

The small ulcers were likewise surrounded by hyperplastic hyperkeratinized squamous epithelium (Fig. 11). In two rats it appeared as though the thickened corneum and superficial malpighian layers were being shed off or pulled off into the lumen. In one animal (Fig. 10) a focus of acute inflammatory cells in the submucosa, and in another edema of the papillae, accompanied these superficial changes, to give evidence that the process was certainly not entirely, and possibly not primarily, a mucosal one.

In animal 41L16 a punched-out ulcer was noted on routine microscopic study of the forestomach, whereas neither gross nor microscopic ulceration was found in the esophagus. In addition there was nearby a verrucous focus in the mucosa formed by hyperplastic cells of the stratum germinativum which grew down to the muscularis mucosae obliterating the submucosal papillae (Fig. 13). The appearance and location of these lesions is reminiscent of those encountered by numerous authors in vitamin A deficiency.

Only one animal in the series exhibited saccular dilatation of the esophagus without demonstrable ulcerative changes in the mucous membrane. Rat 32L61 was peculiar in this respect (Fig. 1) and there is some doubt that ulcerative changes were not present in the mid-esophagus of the animal. Sections of the mid-esophagus showed ad-

vanced thickening and fibrosis of the muscularis. The muscularis at this level was heavily infiltrated with polymorphonuclear leukocytes, eosinophils and lymphocytes, and the muscle fibers were largely replaced by proliferating fibroblasts. Examination of the overlying squamous epithelium revealed thickening but no ulcerative defect.

Somewhat similar changes were noted in the mucous membrane of rat 29L61 (Fig. 7) although no dilatation accompanied them. In the lower esophagus of this animal the squamous lining was in some places atrophic, while in others it was hypertrophied, heaped up and papillary. The rete pegs were separated by delicate edematous connective tissue papillae in which scattered plasma cells and dilated capillaries were noted (Fig. 8).

Two additional animals, 14L51 and 38L16, exhibited definite hyperkeratosis (Figs. 6 and 9). Beneath this, scattered polymorphonuclear leukocytes lay in a slightly edematous submucosa.

Of the remaining five animals, three had unequivocally normal-appearing esophageal mucosae. In the remaining two the mucosa in the lower end was hypertrophied and covered with a stratum corneum equal to one-half the entire mucosal thickness. Kullmann,[4] after study of the rat esophagus, stated that the cornified layer usually comprised one-third of the thickness of the mucosa. For this reason rats 31L61 and 3L3 were considered borderline cases. None of these five animals exhibited submucosal changes.

Group II

A group of 16 rats similar in age, sex and weight to those of group I was placed on the same deficient diet for a period of 5 months. Synthetic vitamins were added to this diet in the proportions noted below * for a period of 1 month, then the animals were sacrificed. The amounts supplied were well in excess of the vitamin dietary requirements for the rat as determined by Richardson, Hogan, Long and Itschner [5] and contained, in addition, nicotinic acid and vitamin K. The rats ate noticeably increased amounts of the vitamin-supplemented diet, and underwent an average weight gain of 76 gm. Prior to the addition of vitamins four animals developed ulcers of the skin at the base of the tail, one an ulcer of the penis, one an ulcer over the shoulder, and one an ulcer of the scrotum. Upon the addition of the vitamins all of the

* The vitamins are expressed in mg. per 100 gm. of diet: α-tocopherol, 5; thiamine hydrochloride, 1.6; riboflavin, 3.2; pyridoxin hydrochloride, 2.4; calcium pantothenate, 2.0; choline chloride, 800; nicotinic acid, 2; synthetic vitamin K, 0.02. Two gm. of cod liver oil per 100 gm. of rice furnished vitamins A and D. Four gm. of salt mixture (no. 2, U. S. P.) per 100 gm. were also added. Merck and Co. kindly furnished the first seven synthetic vitamins.

ulcers but two healed completely and these two nearly healed. When the animals were sacrificed at the end of the experimental period of 6 months (1 month after the vitamins were added) they appeared healthy. Careful examination of all internal viscera showed no abnormality. Microscopic sections of the parotid gland, trachea, kidney pelvis, bladder, esophagus, stomach, forestomach and cecum were made. Three of this group showed slight hyperkeratosis of the lower esophageal mucosa with underlying round-cell infiltration, and one showed similar foci in the forestomach. No ulcers were noted. Six of the group exhibited multiple minute calcium foci in the tubular epithelium of the kidneys. In six the sections were entirely negative. Microscopically there appeared to be nothing specific about the inflammatory changes occurring at the sites of skin ulceration.

Discussion

In reviewing the lesions associated with the various dietary deficiencies one is impressed with the similarity between the lesions here described and those of vitamin A deficiency. The hyperkeratosis associated with esophageal ulcers seems reasonably characteristic of mucosal changes in A deficiency. After a search through a considerable number of articles [6-13] dealing with the experimental pathology of this condition, however, I was able to locate none describing esophageal ulcers and only one mentioning esophageal hyperkeratosis. Tilden and Miller,[14] in a study of the effects of a low vitamin A intake on 11 monkeys, noted 3 in which keratinization of the esophagus was discernible and 1 in which it was questionable. The same condition was reported in 5 of a group of 17 Chinese suffering from a dietary deficiency, most likely A, by Sweet and K'ang.[15] The absence of other more obvious signs of vitamin A deficiency in my animals indicates that a full-fledged deficiency did not exist. Nor would one expect it in animals receiving even a small amount of carrot daily. It is possible, however, that a partial deficiency did exist and that its effects became cumulative over a long period to become manifest spontaneously or as a result of some local phenomenon—in this case mechanical irritation of the ground rice diet.

Denton's [16] descriptions of the lesions of experimental black tongue in dogs set forth some features which my rats exhibited. He characterized the condition of black tongue as a membranous necrosis of the mucosa of the mouth, pharynx, esophagus, intestines, and skin of the scrotum. No oral lesions occurred in my group, but two rats had duodenitis, and a number of rats in group II exhibited skin lesions at the base of the tail and on the scrotum. The focal nature of the ulcera-

tions in my group stands in contrast to the diffuse gastrointestinal inflammation of black tongue.

Erosions of the gizzard in chickens as described by McFarlane, Graham and Hall [17] and by Dam [18] were thought by Almquist and Stokstad [19] to be caused by a deficiency of a fat-soluble factor not identical with any of the known vitamins. Morphologic studies by these authors are not sufficiently detailed to allow a comparison with the lesions of my rats.

In group II of my series it seems significant that no esophageal ulcers were present after the addition of a full vitamin supplement for only 1 month. Also indicative of a more adequate dietary state is the prompt gain in weight and healing of the skin lesions. Except for the cod liver oil, very little in the way of caloric value was added. The finding of hyperkeratotic mucosal changes in the four animals after the addition of vitamin supplements suggests that a deficiency did exist or was developing prior to the addition of the vitamins.

Summary

1. Lesions of the esophagus of the rat, including mucosal ulceration, hyperkeratosis, various stages of inflammation, and dilatation with muscle atrophy and fibrosis have been described.

2. These lesions appear to be of dietary origin.

I wish to acknowledge my indebtedness to Robert McLemore for technical assistance.

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[*Illustrations follow*]

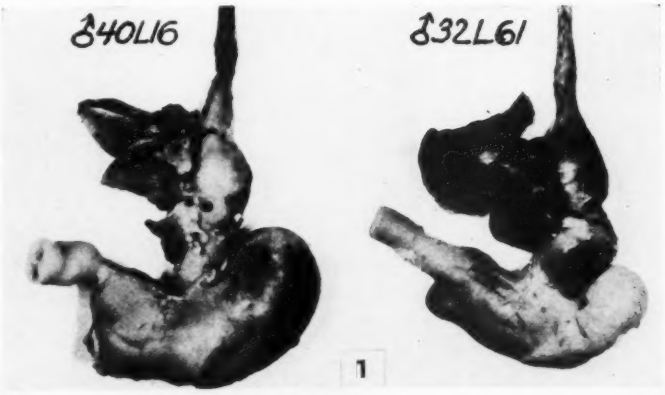
DESCRIPTION OF PLATES

PLATE 96

FIG. 1. Fusiform dilatation of the lower esophagus. Rat 40L16 showed mucosal ulceration, but only hyperkeratosis was noted in rat 32L61. × 1.

FIGS. 2, 3 and 4. Gastro-esophageal junction. There is ulceration with accompanying hyperkeratosis. The lesions found in rats 23L63, 39L16 and 19L51, respectively, are represented. × 2.

FIG. 5. Penetrating ulcer of the lower esophagus in rat 17L63. × 2.



Brown Dietary Ulcers of the Esophagus

PLATE 97

FIG. 6. Rat 14L51. A focus of hyperkeratosis in the lower esophageal mucosa. The rete pegs are thickened. Eosinophils and lymphocytes are scattered beneath the muscularis mucosae. × 48.

FIG. 7. Rat 29L61. A focus of hyperkeratosis from the lower esophagus. Edematous submucosal papillae separate thickened rete pegs. Dark strands on the surface of the keratinized layers are made up of small granules resembling rice. × 48.

FIG. 8. Rat 29L61. A wide zone of edema surrounds a delicate capillary of a papilla. Occasional polymorphonuclear leukocytes and lymphocytes are observed. Fine rice granules can be seen in the thickened stratum corneum. These are smaller than the keratohyaline granules. × 240.

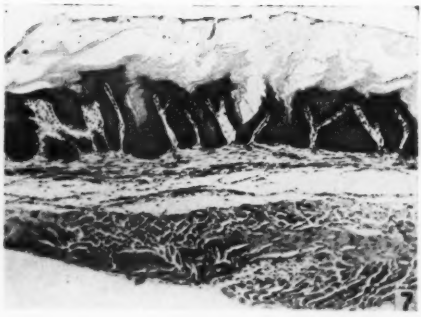
FIG. 9. Rat 38L16. Desquamation of the corneum and superficial malpighian layers caused widespread erosion but no ulceration in the lower esophagus. × 48.

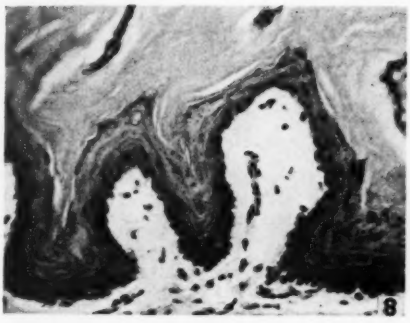
FIG. 10. Rat 39L16. Similar mucosal fragmentation is seen here, but ulceration is beginning and an exudate, made up chiefly of eosinophils, pushes the muscularis mucosae upward. × 48.

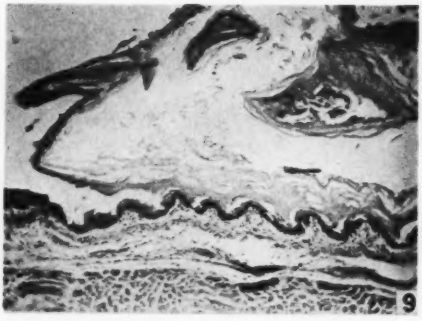
FIG. 11. Rat 24L60. A small pregastric ulcer is seen extending down to the muscularis mucosae. The vacuolar changes in the adjacent squamous epithelium were not conspicuous in other animals. × 48.

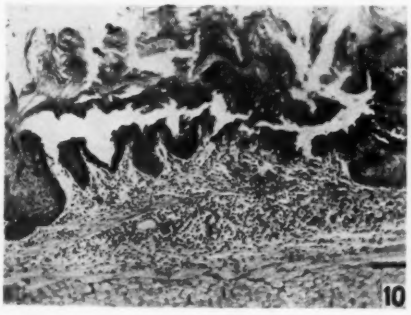
FIG. 12. Rat 19L51. The edges of a large penetrating ulcer are seen, which in its center extends through the muscularis. Necrotic débris, bacteria and rice granules cover the ulcer base. × 48.

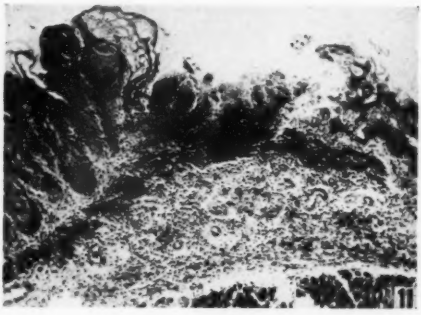
FIG. 13. Rat 41L17. Focal verrucous thickening of the squamous epithelium of the forestomach. Elsewhere in the forestomach was an ulcer similar to that shown in Figure 11. × 48.

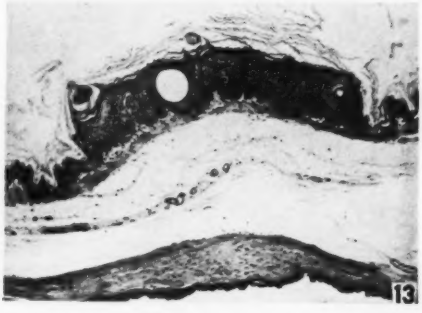












Brown

Dietary Ulcers of the Esophagus

THE CO-INCIDENCE OF PRIMARY CARCINOMA OF THE LUNGS AND PULMONARY ASBESTOSIS *

Analysis of Literature and Report of Three Cases

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A review of the literature on pulmonary asbestosis by Egbert in 1935 resulted in the publication [1] of 25 cases supported by anatomic study, thus reflecting the increasing importance of the disease.

In 1935, Lynch and Smith [2] reported *primary carcinoma of the lungs associated with asbestosis.* Four years later the same authors [3] described 2 additional cases of their own and collected 5 more from the literature,[4-7] bringing the total to 8. A recent survey revealed 8 more cases, which are presented in Table I. Three more instances are contributed in the following report, thus bringing the total number to 19.

Pulmonary asbestosis has been diagnosed in this laboratory according to the following criteria:

1. Fibrosis of the lung.
2. Presence of asbestosis bodies.[8, 9] Kühn [10] presented photographs of asbestosis bodies obtained by the electronic microscope, enlargement × 20,800.

Reports of Cases

Case 1

The patient (autopsy no. 1705), a white male, 45 years old, was exposed to dust in an asbestos factory for 5 years. For at least 1 year before his death he had complained of gradually increasing weakness and dyspnea. This forced him to stay in bed for 6 weeks prior to his admission to the New Haven Hospital. The clinical impression at this time was diaphragmatic pleurisy and compression of the lung on the right side, possibly tuberculous in origin. Generalized arteriosclerosis and paroxysmal tachycardia were found as complicating factors. The patient expired after a short stay in the hospital.

Only those portions of the post-mortem findings important for this report are included.

Gross Description of the Lungs. Each lung weighed 700 gm. The surface of the right lung was covered by white, firm adhesions which bound it to the parietal pleura. Only a few small areas of the visceral pleura were free from adhesions. These areas were pink, mottled with the usual amount of black subpleural pigment. The diaphragmatic surface exhibited a large number of firm, white, opaque nodules, 2 to 5 cm.

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TABLE I

Collected Cases of Primary Carcinoma of the Lung Associated with Asbestosis

Number and author	Year	Sex and age	Occupation in asbestos industry	Duration of exposure	Freedom from exposure before death	Nature of tumor	Primary site	Metastases
* 1. Lynch and Smith[2]	1935	M., 57	Weaver	21 yrs.	4 mos.	Squamous cell	Right lower lobe	Many nodules in right lower lobe
* 2. Gloyne[5]	1935	F., 35	Spinner	8 yrs.	9 yrs.	Squamous cell	Right upper lobe	Pleura
* 3. Gloyne[5]	1935	F., 71	Mattress and opening department	19 mos.	15 yrs.	Squamous cell	Right lower lobe	None
* 4. Egbert and Geiger[4]	1936	M., 41	Weaver	17 yrs.	2 yrs.	Glandular	Left lower lobe	Widespread
* 5. Gloyne[6]	1936	M., 59	Packer	10½ yrs.	? mos.	Oat cell	Left lower lobe	Left upper lobe; pleura
* 6. Nordmann[7]	1938	F., 35	Spinner	7 yrs.	9 yrs.	Squamous cell	Left lower lobe	Liver, kidney
* 7. Nordmann[7]	1938	M., 55	Pre-spinning assembly	7 yrs.	12 yrs.	Squamous cell	Left lower lobe	Widespread
* 8. Lynch and Smith[3]	1939	M., 50	Weaver	13 yrs.	3 yrs.	Squamous cell	Right lower lobe	Pleura, mediastinal lymph nodes
9 and 10. Koelsch[11]	1940	No details known. Oral communication of Domenici, quoted by Koelsch.						
11. Linzbach and Wedler[12]	1941	No details known. Oral communication of Bohne, quoted by Linzbach and Wedler.						
12. Linzbach and Wedler[12]	1941	M., 61		At least 3 yrs.	Not exactly known	Squamous cell	Right lower lobe	None

TABLE I—(*Continued*)

Number and author	Year	Sex and age	Occupation in asbestos industry	Duration of exposure	Freedom from exposure before death	Nature of tumor	Primary site	Metastases
*13. Holleb and Angrist[13]	1941	M., 52	Pipe insulator	25 yrs.	9 wks.	Squamous cell	Right upper lobe	Mediastinal nodes, kidney, adrenal
*14. Holleb and Angrist[13]	1941	M., 58	Pipe insulator	25 yrs.	10 yrs.	Oat cell	Right lower lobe	Widespread
15. Desmeules and others[14]	1941	M., 57	Machine adjuster	25 yrs.	1 mo.(?)	Alveolar cell	Left lung	Pleura
16. Desmeules. and others[14]	1941	M., 50	Bagger	22 yrs.	4 mos.	Squamous cell	Right lung	Pleura
17. Homburger	1942	M., 45	Not known	5 yrs.	1 yr.	Squamous cell	Right lung	Diaphragm
18. Homburger	1942	M., 43	Not known	20 yrs.	17 mos.	Anaplastic	Left lower lobe	Pleura
19. Homburger	1942	F., 49	No known contact with asbestos	Not known	Not known	Squamous cell	Right lung	Liver, adrenal, stomach, hilar lymph nodes

* Included in previous tabulations by Nordmann, Angrist and Holleb.

in diameter with scattered zones of necrosis. Similar masses were attached to the parietal wall of the pericardium. The lung was firm and only slight crepitation could be elicited. This was particularly true at the angle between the diaphragmatic and the mediastinal surface. On section this latter portion was white and opaque. Smaller nodules were seen in the lung parenchyma near the interlobar fissures. The color of the remaining lung parenchyma was mottled dark red and black. The cut surface was moist and exuded red frothy fluid upon slight pressure. Some of the black zones were firmer than the remaining parenchyma. In the left lung similar black, firm, fibrous nodules and a red, moist cut surface were encountered. There were no white nodules in this lung.

Microscopic Description. The white, firm nodules seen grossly in the right lung proved to be squamous cell carcinoma with mitoses and pearl formation. The tumor involved the walls of the bronchi. It extended into the pleura where it was associated with much fibrous tissue and marked thickening. The lung not involved by tumor was well preserved. Some interstitial fibrosis was noted in these areas. The alveoli were filled with mononuclear cells. *Asbestosis bodies* were present in the lumina of alveoli, in the alveolar walls, in the tumor proper and in the interstitial fibrotic tissue of the lung as well as in the pleura. There was a chronic bronchitis but the epithelium of the bronchi was essentially unaltered. The exact point of origin of the tumor was not determined (Figs. 2 and 3).

Anatomic Diagnosis. Pigmentation and fibrosis of lungs (histologically, asbestosis); bronchiectasis; fibrous pleural adhesions; squamous cell carcinoma (probably carcinoma of bronchus) involving right lung, pleura, pericardium and thoracic wall; cardiac hypertrophy and dilatation; passive congestion of viscera; emaciation. Subsidiary: Cyst of thyroid gland; adenoma of pancreas.

Case 2

The patient (autopsy no. 3841) was a white male, 43 years old, who had worked in an asbestos factory for 20 years. Asbestosis bodies had been found in his sputum 17 months prior to his death. For several years before his death he had complained of pain in the back, radiating to the lower limbs. This was shown to be associated with rotation of the first lumbar vertebra and sacralization of the fifth lumbar vertebra. There had been also an incomplete fracture of the left femur. Generalized mottling throughout both lungs was observed at this time. When the patient began to complain of shooting pain in the lower back, ascending to the midback and radiating to the left side of the chest, further roentgenograms were made of the chest and a circumscribed shadow in the left lower lobe was interpreted as a lung tumor. In retrospect it was possible to see this shadow on earlier films. The tumor displaced the left main bronchus and the esophagus to the right. Exploratory thoracotomy and biopsy established the diagnosis of anaplastic carcinoma. The

patient became cyanotic after operation, developed pneumonia of the left upper lobe and expired.

Clinical Diagnosis. Pneumonoconiosis (asbestosis); lung tumor (left lower lobe) with pressure on spinal root or metastases to spine.

Gross Description of the Lungs. The right lung was normal in size and shape. It was remarkably heavy, only slightly crepitant and held its shape when removed from the body. It was covered by a uniformly thickened, gray and translucent pleura. The cut surface was relatively smooth, finely granular, pale, slate-gray tinged with pink and mottled due to the presence of innumerable small, slate-gray polygonal areas bounded by gray-white connective tissue septa. In the lower lobe these areas were crepitant; the surface had a gray, more homogeneous appearance and was still firmer than that of the upper lobe. The walls of the smaller bronchi and bronchioles were thickened; a rather marked reddening of the mucosa of the larger bronchi was evident. In the posterior part of the left lower lobe a firm, irregular mass, measuring 5 by 6 by 7 cm., was encountered. Immediately beneath it lay the 8th and 9th intercostal nerves. It projected posteriorly and also medially from the surface of the lung. Posteriorly, the visceral and parietal pleurae were thickened and fused over the tumor in an area about 4 cm. in diameter, where the mass was covered only by delicate areolar tissue. Medially it projected a distance of several centimeters into the mediastinum, being adherent to the descending aorta and displacing the aorta and esophagus to the right. The left vagus nerve was embedded in the tumor mass for a distance of about 3 cm. at a point well below the cardiac plexuses.

No other mediastinal structures appeared to be involved. There were several large, soft mediastinal lymph nodes which, on section, presented a brownish black surface.

The cut surfaces of upper and lower lobes of the left lung resembled those of the right lung. The cut surface of the tumor was gray-white and marble-like with scalloped edges. It was closely associated with, and in one place apparently involved, the wall of a branch of the left main bronchus. Careful examination revealed no evidence of extension or metastasis to the nearby vertebrae or ribs.

Microscopic Description. Diffuse fibrosis was present throughout the lungs, not only in the peribronchiolar and perivascular regions but also in many of the alveolar septa. The alveoli were lined with cells which were almost uniformly swollen, prominent and in many cases detached from the wall. Many of the alveoli contained typical *asbestosis bodies* (Fig. 1). A few of them lay within the peribronchial or perivascular lymphatic tissue but the majority were in the alveolar spaces. They

were rarely free, but usually surrounded and sometimes partially phagocytized by large mononuclear cells and multinucleated giant cells. Scattered anaplastic cells with atypical hyperchromatic nuclei and abundant cytoplasm were present in the tumor mass. Numerous mitotic figures were seen. In other sections the cells were in cords arranged on a scanty connective tissue stroma. There was a tendency to form alveoli. In one area the tumor cells had grown in sheet-like masses, suggesting an epidermoid carcinoma. Branches of the vagus nerve were found firmly embedded in neoplastic tissue and the perineurium was invaded by carcinoma cells. Rare small asbestosis bodies were seen in the sinuses of a tracheobronchial lymph node in addition to many phagocytes loaded with anthracotic pigment (Fig. 6).

Anatomic Diagnosis. Pulmonary asbestosis; carcinoma of lung (left lower lobe), compressing 8th and 9th intercostal nerves and surrounding and compressing left vagus nerve; scar of recent thoracotomy; serofibrinous pleurisy (left). Subsidiary: Fibrous apical scars (bilateral); fibrous pleural adhesions (bilateral).

Case 3

The rather incomplete history of this white female (autopsy no. 5443), 49 years old, failed to reveal contact with asbestos. Nineteen months prior to death she began to note shortness of breath and dry cough. There was scanty mucoid sputum. As her condition grew worse x-ray examinations led to the diagnosis of pneumonia and pleurisy on the right. Thoracic taps revealed right hemothorax. Some days prior to death, examination of the thorax showed displacement of the heart to the left and dullness over the right chest except for paravertebral tympany. There was also wheezing anteriorly and posteriorly. Straw-colored fluid was repeatedly aspirated from the right chest.

Clinical Diagnosis. Carcinoma of the right lung with metastasis to the liver.

Gross Description of the Lungs. The right lung weighed 1050 gm. The upper lobe was atelectatic and covered by thick, firm, fibrous adhesions. Crepitation was impaired in the peripheral portions of the middle and lower lobes.

The lungs were cut on a mechanical slicer into slabs 2.5 cm. thick. The medial third of the upper lobe was replaced by firm white nodules of neoplastic tissue in which compressed blood vessels and bronchi were still recognizable. In the upper portions of the middle lobe the tumor extended along the bronchi and blood vessels leading to the periphery, and the mediastinal lymph nodes were enlarged by tumor. The lung parenchyma, where free of tumor, was pale gray. Its interstitium was markedly thickened, gray and translucent. The consistency was rubbery and in the upper lobe there were some yellow areas of localized softening about 1 cm. in diameter. The bronchi,

carefully examined, were found to be free of intraluminal obstruction. The mucosa was red and swollen. No ulceration could be seen. The walls of several bronchi were thickened and the lumina reduced. Analogous changes were found in the intrapulmonic vessels on this side.

The left lung weighed 360 gm. Its pleural surface was thin and translucent and free of changes. The parenchyma was air-containing; the color was gray in the upper and red in the lower lobe. There was the usual pattern of anthracotic pigment in the pleura and, on section, the parenchyma was dry and only a few droplets of blood escaped from the section of the lower lobe. All the bronchi had the usual caliber; their mucosa was intact and pink. There was no change in the blood vessels of this side. The hilar lymph nodes were free from tumor.

Microscopic Description. The tumor in the right lung was composed of dense nests of squamous cells surrounded by fibrous connective tissue. These tumor cells varied in size and had pyknotic nuclei. Many were in mitotic division. The cytoplasm was clear and pale.

The neoplastic tissue had replaced the lung parenchyma. There were also large areas of round cell infiltration and foci of breakdown of the tumor. The pleura was locally thickened by dense fibrous tissue. There was also interstitial fibrosis. There was a moderate amount of anthracotic pigment. Adjacent to the tumor the lung was atelectatic. In some areas, however, the alveoli were dilated and filled with small and large round cells or amorphous pink-staining material. Rod-shaped brown deposits were present in the interstitial tissue and also in some of the alveoli. These had the appearance of *asbestosis bodies* (Fig. 4). They were scattered throughout the lung parenchyma near the tumor and also in more remote regions of the lung.

Most of the bronchi showed inflammatory changes with round cell infiltration in the submucosa. The epithelium was of the usual type and in some places was lifted from the limiting membrane by the round cells. Most of the lumina contained similar mononuclear cells. In one instance remnants of bronchial epithelium were seen in the center of a tumor nodule. Here the lung parenchyma was entirely replaced by dense fibrous tissue which constituted the stroma of the squamous cell carcinoma. Pearl formation was observed. There was also a purulent polymorphonuclear exudate in some of the bronchi and in some of the larger bronchioles.

Anatomic Diagnosis. Pulmonary asbestosis (microscopic); purulent bronchitis and bronchiolitis; bronchogenic carcinoma (right lung); metastasis to hilar and preaortic lymph nodes, liver, adrenal and gastric mucosa; infarct in spleen. Subsidiary: Uterine fibroids; healed mitral endocarditis; nevus on abdominal skin.

Discussion

In 4137 autopsies from 1918 to 1938 there were 45 cases of pulmonary carcinoma in this laboratory,[15] an incidence of 1.08%. Asbestosis was diagnosed 8 times, silicosis 17 times. Pulmonary carcinoma was found in 4* of the 8 instances of asbestosis and twice in the 17 silicotic cases.

A similar coincidence of silicosis and cancer was found by Klotz[16] (8%), but Vorwald and Karr[17] could not confirm this. Because of the small series of cases, the value of these data is limited. Lynch and Smith[2] suggested that asbestosis is a predisposing factor in carcinoma of the lung. They stated that advanced asbestosis "may lead to bronchial epithelial metaplasia of a type encountered in other locations where cylindrical epithelium may give rise to squamous cell carcinoma," a point of view also shared by Linzbach and Wedler.[12] They cited the case of a negro who was thought to be asthmatic and who apparently died of heart failure. At autopsy pulmonary asbestosis, chronic bronchitis and bronchiectasis, acute lobar pneumonia and pulmonary atheromatosis were found, together with hypertrophy and dilatation of the heart. In the bronchi "foci of transformation of undenuded epithelium into stratified squamous form were found." It seems questionable whether this statement is of significance as an indication that asbestosis predisposes to carcinoma. During a study on bronchial metaplasia in this laboratory, metaplasia of the bronchial epithelium was found in only one instance of pneumonoconiosis (case 5549, Fig. 10).

Metaplastic changes of bronchial mucosa were observed four times in a series of 44 consecutive autopsies, studied with special care from this point of view. Such metaplastic changes, illustrated in Figures 5, 7, 8 and 9, were seen in cases with various pulmonary lesions. None of these four cases presented any deposits of foreign material in the lungs.

These facts seem to indicate that neither from statistical calculations nor from purely morphologic studies is there any reliable answer to the question whether pulmonary asbestosis has to be considered as an etiologic factor in pulmonary carcinoma.

Summary

A review of the literature on the association of pulmonary asbestosis and carcinoma revealed that there are at least 19 known cases (including the 3 herein reported) of asbestosis associated with primary pul-

* One of these cases was published previously by Egbert and Geiger.[4]

monary carcinoma. In this laboratory the association of the two conditions is remarkably high. In 8 cases of asbestosis there were 4 instances of primary pulmonary carcinoma.

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[*Illustrations follow*]

DESCRIPTION OF PLATE

PLATE 98

FIG. 1. Case 2 (A. 3841). Asbestosis bodies and phagocytes in pulmonary alveolar lumen. × 875.

FIG. 2. Case 1 (A. 1705). Nests of carcinoma cells, pulmonary fibrosis and numerous asbestosis bodies. × 220.

FIG. 3. Case 1 (A. 1705). Asbestosis bodies among carcinoma cells in lung. × 615.

FIG. 4. Case 3 (A. 5443). Asbestosis bodies and carcinoma cells in lung. × 395.

FIG. 5. White male, 36 years old (A. 5631). Death by violence. Pulmonary fibrosis. Bronchial metaplasia. × 220.

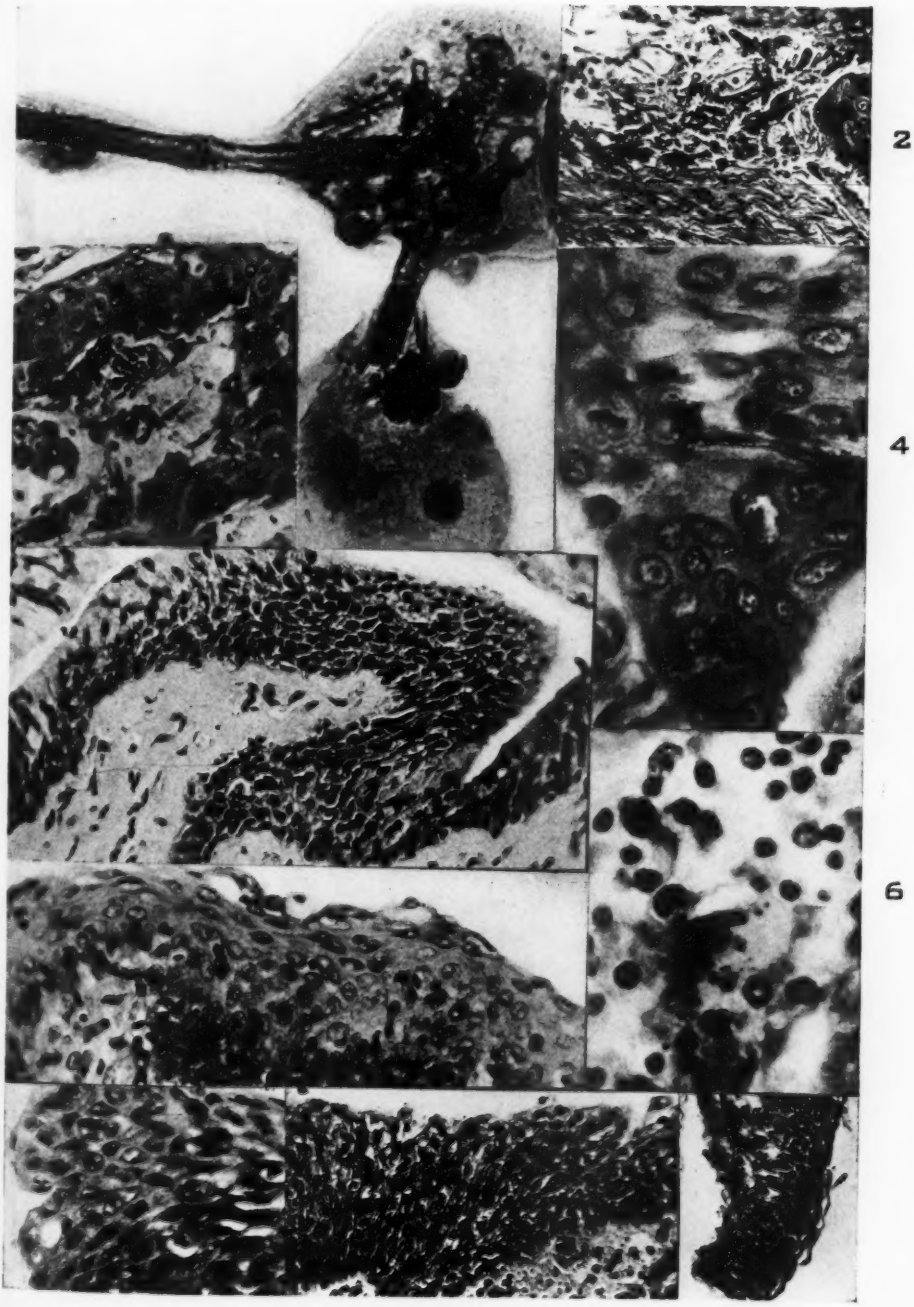
FIG. 6. Case 2 (A. 3841). Asbestosis body in sinus of peribronchial lymph node. × 655.

FIG. 7. White male, 57 years old (A. 5685). Healed endocarditis. Generalized arteriosclerosis. Pulmonary congestion. Bronchial metaplasia. × 370.

FIG. 8. White male, 40 years old (A. 5475). Caseous and ulcerative pulmonary tuberculosis. Bronchial metaplasia. × 370.

FIG. 9. White male, 66 years old (A. 5545). Pulmonary thrombo-arteritis, focal pneumonia. Bronchial metaplasia. × 220.

FIG. 10. White male, 43 years old (A. 5549). Chronic pulmonary abscesses, bronchiectasis, organizing pneumonia. (Questionable anthracosilicosis.) Bronchial metaplasia. × 220.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LOCAL MYELOPOIESIS IN MYELOID LEUKEMIA*

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Two explanations have to be considered for the genesis of extramedullary myelopoiesis. First, the hematopoietic foci may have developed *in situ* from local cells through metaplasia or prosoplasia by local transformation of unspecific cells into immature blood cells, which secondarily may enter the local vessels, thus reaching the circulating blood; second, production of the immature cells may have occurred in the bone marrow, with transportation by the blood stream to the involved organs, and formation of the foci by invasion of the perivascular tissue. Bloom [1] has suggested calling the first mechanism "heteroplastic" and the second "homoplastic." These short and significant terms are rarely used in the literature.

In general, the first theory is known by the phrase, extramedullary *myelopoiesis by local origin*. For the second, which has a basic similarity with the formation of deposits by malignant tumors, a special term, *colonization*, has been employed instead of using the term metastasis, to avoid a not fully proved analogy between leukemic blood cells and malignant tumor cells.

The mechanism of colonization itself shows certain remarkable differences from metastasis. In the choice of the host organ in which invading tumor cells settle and proliferate, in some special cases, biological affinity plays a deciding rôle. The ovary, for instance, is almost immune against cervical carcinoma growing nearby, but offers a particularly favorable soil for the cells of carcinoma of the stomach of distant origin. In general, in lymphogenic deposits, localization depends on mechanical conditions and topographical relations to the direct lymph flow. In hematogenic deposits, it is due to filtration of the tumor cells through narrow capillaries, as, for instance, in the pulmonary deposits of chorionic epithelioma. In "colonization" we see a definite predilection for certain organs. The immature blood elements are present in the circulating blood in numbers incomparably higher than malignant cells of any neoplasm ever are. They reach every organ of the body in a relatively high number, but settle in special organs only. This preference cannot be explained by narrow filtering capil-

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laries, since these are present in all organs. Also, the lymphatic connection exerts no influence, since colonization is accomplished only by the blood stream.

If we accept the hypothesis of colonization, we must face the fact that the organs selected by the immature blood cells for colonization, as the spleen, liver and, eventually, lymph nodes, are at the same time the organs of fetal hematopoiesis. This coincidence can only be explained by the auxiliary hypothesis, that in consequence of their function in fetal life, the tissues of these organs in extra-uterine life still retain a certain affinity for some elements of the blood. Special mechanical conditions may play a supporting rôle. Jaffé,[2] in his paper on extramedullary myelopoiesis in anemic mice, explained the myeloid foci he found in the liver either by colonization due to reduced speed of the blood in the wide capillary net of the liver or, possibly, to impaired circulation in the sinusoids, the lumen of which is reduced by Kupffer cells which are swollen in consequence of phagocytosis of red blood corpuscles. These mechanical conditions are responsible for the retention and the concentration of the circulating myelocytes. But in the same paper he stressed the relationship of Kupffer cells, sinus endothelial cells and reticulum cells with the elements of the bone marrow, and pointed out that these cells represent "familiar surroundings" for the circulating immature blood cells and thus facilitate their settling. Maximow [3] described similar intravascular myeloid foci in the liver which he traced to stem cells deposited by the blood stream. However, such observations can serve only to explain the accumulation of myeloid cells inside of the sinusoids and contribute no clue for the histogenesis of the myeloid foci in the periportal fields.

In animal experiments the time relation plays an important rôle in making colonization probable. When the eosinophile cells appear in the tissues a few hours after a marked decrease in the same cells in blood, as, for instance, in the experiments carried out by Opie [4] and by Homma,[5] migration from the blood into the tissues or colonization is very probable. Most of these experiments, however, concern mature granulocytes only. For mature granulocytes active immigration by ameboid motility can be generally considered, whereas for immature ones only passive locomotion can be admitted. Moreover, eosinophile granulocytes, even when reaching full maturity, possess, as Sabin [6] and Ringoen [7] have pointed out, only a reduced active motility. However, in the experiments in which local accumulation of eosinophils by administration of bacteria or foreign animal proteins is accomplished, colonization by circulating myelocytes caused by chemotaxis seems admissible. The theory of colonization has found many authors to sup-

port it, from Ziegler [8] and Helly [9] in 1906 to Jaffé [2] in 1921, Lang [10] in 1926, and Maximow [11] in 1927.

Local origin of extramedullary foci was described in 1905 by Pappenheim,[12] in 1916 by Herzog,[13] in 1922 by Dieckmann,[14] in 1931 by Naegeli,[15] in 1926 by Ssyssojew,[16] and by many others. It requires a different interpretation when it occurs with deficient erythropoiesis of bone marrow than when found with pathologically exuberant leukopoiesis in leukemia. In the first case it may be explained as compensation for the insufficient activity of the involuted bone marrow; in the second, the pathological agent which caused the proliferation of the bone marrow may have stimulated also the extramedullary undifferentiated elements of the mesenchyma, as far as they are apt and ready for metaplastic transformation into immature blood cells. In the first group the extramedullary myelopoiesis is an attempt at healing; in the second, it is a part of the pathological process itself. Changes of the first type were described by Brannan [17] as fairly common in certain anemias of infancy and childhood. Erythropoietic foci are found not only in the organs of fetal hematopoiesis, such as liver, spleen, and lymph nodes, but large red masses of proliferating hematopoietic tissue can develop in the hilus of the kidneys and the falx cerebri.

It is interesting that in some of these cases the thymus, which physiologically is concerned with the production of lymphocytes only, changes into erythropoietic tissue—an ambiguity of function which parallels the activity of the red lymph nodes. In secondary anemia of adults, for instance in protracted bleeding after abortion, Brannan [17] found erythropoietic tissue in organizing thrombi of the broad ligament.* These findings, together with the incidental formation of bone tissue in calcified thrombi as described by Wydler,[22] prove the great and manifold prospective potencies of the fibroblasts which develop from the vascular wall and invade and organize the thrombi. The compensatory character of the extramedullary erythropoiesis becomes evident by the fact that in animal experiments it is demonstrable only in anemia provoked by agents injurious to the bone marrow, but not in cases where such factors are missing. A toxic incentive, which acts specifically on cells endowed with hematopoietic potencies, is yet to be isolated, as Lang [23] pointed out. It seems that, among the various types of interstitial tissue, the fat tissue possesses a special capacity for metaplastic change into hematopoietic tissue. Petri,[24] Wasser-

* Cone [18] found in 68 of 250 necropsies bone marrow filling the intercostal veins which duplicated the bone marrow in the ribs. Like Lubarsch,[19] Bunting [20] and Maximow [21] in earlier observations, he attributed this bone marrow to embolism of myelogenous elements.

mann,[25] Jordan [26] and others have described multiple, newly formed, small hematopoietic foci in the fat tissue of various organs in cases of polycythemia, carcinosis and endocarditis. The hematopoietic islands develop from undifferentiated reticular elements which persist from fetal life in the fat tissue and may be classified among the so-called indifference zones of Schaper and Cohen.[27] Such pluripotent elements of low differentiation probably serve for the frequent changes between fatty and functional bone marrow. When, physiologically or pathologically, cells of these two types replace each other, it is not only done by reduction of one and *ex vacuo* proliferation of the other, but also by differentiation of these reticular pluripotent elements in one or the other direction and subsequent proliferation.

For the cells which give origin to extramedullary myelopoietic foci in leukemia, some authors have developed a broad conception which goes back to pluripotent qualities of embryonal mesenchyma. Dieckmann,[14] in his paper on extramedullary hemopoiesis, has discussed this theory, which finds support in pathological investigations of Hueck [28] and in experiments carried out by Busse,[29] Maximow [3] and others. Particularly, the observation that unspecific connective tissue, for instance from the heart valves, in tissue cultures may produce granulocytes or lymphocytes if stimulated, extends the faculty of hematopoiesis, as far as white cells are concerned, to a large field of unspecific mesenchyma. Modern conceptions in embryology, as represented for instance by Gruenwald,[30] are in favor of very little restriction in prosoplastic potencies, and some recent findings in pathology cannot be explained satisfactorily without such embryological conceptions. Heterotopic endometrium, as encountered in many locations within the peritoneal cavity far from the uterus, finds its explanation by Heim's [31] theory that primarily not only the müllerian duct but the whole coelomic lining possesses the capacity to form endometrium. This potency is physiologically abolished everywhere outside of the müllerian duct and may be pathologically preserved in any portion of the peritoneal lining. If aroused later by some stimulus, local prosoplastic differentiation can give origin to islands of heterotopic endometrium, *i.e.*, of peritoneal endometriosis.

The investigators who support a more restricted and specific origin of local myelopoiesis agree that in general the distribution of the foci shows a close relation of the myelogenous tissue to the blood vessels. As far as such observations concern the development of leukemic blood cells from endothelial elements, particularly of the liver and spleen, they harmonize with the conception of those who adhere to the theory that one group of white blood corpuscles—the monocytes—physiologi-

cally develop from endothelial elements of liver and spleen. Hitherto, two types of white blood cells have been observed developing incidentally from Kupffer cells: the megakaryocytes and the monocytes. Downey, Palmer and Powell [32] have described a case of atypical myelosis in a white woman of 56 years, with from 1900 to 3300 white blood cells per cubic millimeter, increasing to 10,400 1 month after splenectomy. Specimens obtained from the liver during splenectomy showed megakaryocytes developing directly from the reticular stellate cells of the liver sinusoids without going through the polykaryocytic stage. Bloom [1] succeeded in provoking similar changes artificially in rabbits by injections first with lithium carmine and subsequently with *Bacterium monocytogenes*. Transformation of Kupffer cells into monocytes was observed by Jaffé [33] in a Chinese male of 28 years who had developed an acute monocytic leukemia (3,650 white blood cells with 28 per cent monoblasts and 41 per cent monocytes) with hemorrhages of lips and gingiva and hemorrhagic extravasations in the mesentery, the myocardium and the pleura. Many of the monocytes of the blood gave a positive oxidase reaction. In the sections of the liver, the Kupffer cells, though still sessile and stellate, were packed with oxidase granules, as were some of the endothelial cells of the splenic sinuses. I have had the opportunity to observe a case which showed this change clearly.

Report of Case

E. R., a white man, 37 years old, suffered an attack of pain in the right lower quadrant of the abdomen 3 months before admission. A physician made a diagnosis of acute appendicitis, but blood count indicated a severe anemia, which contraindicated operation. The patient recovered from the attack but developed increasing weakness. Three days before admission he noted a dull, aching pain throughout the left side of the abdomen, which persisted. At the same time he started to bleed from the nose. He developed shortness of breath, which became progressively worse. Pulse was 120; respiration, 28; blood pressure, 100/60 mm. of Hg. Petechiae appeared in the left upper lid, and on the soft and the hard palate. On the tongue, at the tip, a 3 cm. irregular, firm, reddish yellow, tender mass was visible. Small, hard lymph nodes were palpable in the left supraclavicular fossa and in both axillae. The heart was slightly enlarged to the left. The spleen became very tender and was markedly enlarged. The liver also was tender and palpable two fingersbreadth below the right costal margin. The blood showed: hemoglobin, 37%; red blood cells, 1,900,000; microcytosis; white blood cells, 35,000 increasing to 64,000; neutrophile polymorphonuclears, 4%; lymphocytes, 13%; monocytes, 61%. The urine was cloudy; albumin, 4 plus; no sugar; many leukocytes and occasional red blood cells. Kahn and Wassermann tests were negative. The patient received several blood transfusions but became increasingly weaker and died 1 week after admission.

According to the family physician, 4 months before admission the blood findings were: hemoglobin, 82%; red blood cells, 3,400,000; white blood cells, 3,200; polymorphonuclears, 21%; eosinophils, 2%; bandforms, 2%; mononuclears, 5%; lymphocytes, 70%; blood platelets, 406,000; clotting time, 2 min.; bleeding time, 4

min. The stomach contents contained no free acid. Purpuric spots extended over the anterior surface, particularly of the legs. In spite of the predominance of the lymphocytes and because of the enlargement of spleen and liver, the marked anemia, and the mounting leukocytosis at this time, a subleukemic myelosis was diagnosed.

Autopsy. The following diagnoses were established by the autopsy: acute fibrino-hemorrhagic pericarditis; splenomegaly of 1470 gm.; subacute fibrinous perisplenitis and numerous anemic and hemorrhagic infarcts; myeloid infiltrations in heart, liver, kidneys and the distal half of the tongue; bilateral bronchiectasis; hemorrhages of both pleurae, of the mucosa of the stomach, of the epicardium, and of both kidneys in the subcapsular region. Microscopically the infiltration, which was of the same character in the enlarged lymph nodes, in the septa of the myocardium, in the pulp of the spleen, and in the periportal fields of the liver, consisted mainly of immature monocytes and monoblasts as well as of some myelocytes, metamyelocytes and polymorphonuclears. Many of the granulocytes were eosinophils. Most of the endothelial cells of the liver sinusoids presented a swollen and enlarged protoplasm. Some were of rounded contour and partially detached. Upon staining for oxidase the protoplasm of these cells presented numerous greenish black granules. Transitions from the fixed sinus endothelium cells into mononuclears were seen in great number, many of which were freely floating and filled the lumen of the sinusoids (Figs. 1 and 2).

This and Jaffé's [33] case prove that monocytes can develop from endothelial cells, at least in monocytic leukemia. For the normal this has been repeatedly suggested since the classical observation by Mallory,[34] who, as early as 1898, described desquamation of histiocytes from the vessels in the liver, spleen and lymph nodes of a patient with typhoid fever. Aschoff and Kiyono,[35] who distinguished two types of blood monocytes by the observation that after injection of collargol or lithium carmine some of the circulating monocytes do and some do not store the colloids, called the storing ones "histiomonocytes" and traced them back to desquamated reticulo-endothelial cells. Schilling,[36] in 1926, after having observed in endocarditis lenta as well as in mononuclear leukemia a continuous series of transitions between histiocytes and monocytes, established a triple system of white blood cells by adding to the two groups of granulocytes and lymphocytes the monocytes as a third independent group; that is, granulocytes originating from bone marrow, lymphocytes from the lymphatic tissue and monocytes from the reticulo-endothelial system. Similar origin of the monocytes has been assumed by Schittenhelm and Erhardt,[37] and by Büngeler.[38] On the other hand, Maximow [39] traced the monocytes back

to polyblasts and Bloom [40] suggested that they originate from lymphocytes only. He called the monocytes the physiological polyblasts of the blood, and described their development in the blood stream, especially in the sinuses of liver and spleen, by individual transformation of lymphocytes. Cases like Jaffé's [33] and mine prove the possibility that endothelial cells, or, more exactly, that Kupffer cells can be transformed into monocytes, at least in pathological conditions. Similarly, just as the granulocytes in cases of leukemia are different from the normal granulocytes, not only by being immature but by possessing some biologically obvious but morphologically latent characters by which they are related to neoplastic cells and differ from normal ones, so it is also likely that the monocytes of monocytic leukemia are pathological cells of different biological character compared with the physiological monocytes. This is made probable by the following microscopical observation. Whereas the monocytes of the normal blood, even when examined with the specially refined and accurate methods of McJunkin,[41–43] rarely present a few oxidase-positive granules only, in monocytic leukemia frequently almost all of the blood monocytes are loaded with great numbers of such granules. It seems that the production of oxidase-positive granules is a component of the pathological process, in the sense that a character which normally is present in low degree in some monocytes becomes strongly developed in all of them. This conception agrees with the findings of McJunkin, who proved the presence of some oxidase-positive granules in some of the Kupffer cells.

More frequent than the observations concerning the endothelial elements are those which prove the transformation of fixed elements of undifferentiated character, located in or adjacent to the wall of small blood vessels, into immature white blood corpuscles. This metamorphosis may pass through the phase of basophile hemocytoblast or may skip it, as observed by Bloom,[40] Maximow [3, 11, 39, 44] and Lang.[23] The original cells are classified as adventitial histiocytes or, under the older term, as clasmatocytes or as perivascular reticulum cells. The low differentiation and high prosoplastic potencies of these cells have been definitely proved, although certain fargoing theories, which are based upon these qualities, failed to meet general recognition, as, for instance, the hypothesis of Bostroem [45] which attempted to interpret the generalization of chorionic epithelioma not as metastases but as pluricentric transformation of adventitial elements in chorio-epitheliomatous tumor cells.

However, the occurrence of perivascular myeloid foci can be explained without the conception of special adventitial cells prone to undergo myelogenous metaplasia. Lang [23] offered the theory that the

incentive for the transformation, which is administered to the tissues by the circulating blood, is present in the highest concentration in and next to the vascular wall, and that as a result of this local high concentration the metaplastic immature blood cells are more frequent in this location. In spite of extensive experimental work, the question of the origin of the artificially provoked extramedullary myelopoiesis in animals has not been settled. Barnes and Sisman,[46] who, in co-operation with Furth,[47] successfully established criteria for differentiating myeloid leukemia from nonmalignant extramedullary myelopoiesis in mice, admitted that the study of their material did not aid in solving the problem whether myelopoiesis takes its origin in the spleen and liver or originates from primitive cells that are carried to these organs by the blood stream—that is, by colonization.

For extramedullary myelopoiesis in man even the careful analysis of numerous cases has not yielded a final solution, and Boyd [48] wrote in his textbook that there is no more histological evidence for the one view than for the other. It seems that colonization is easier to prove than local origin. The high frequency in bone marrow and in blood, the small perivascular foci in liver, spleen and lymph nodes and the histological picture of numerous immature blood cells migrating through vascular walls make colonization seem probable. It has to be admitted that the finding of blood cells in the vascular wall does not prove in which direction they migrate, whether from the lumen towards the perivascular tissue or in the opposite direction. The same doubt can be raised against sweeping conclusions based on higher concentrations of the immature blood cells in the vessels of the organs which harbor the extramedullary foci (observations by Lang,[23] Barnes and Sisman,[46] Askanazy,[49] Herzog [50]). The cells may accumulate in these vessels before settling in the tissues, or they may have entered the vessels after originating in the local foci. If they are found in a typically restricted part of the vascular wall only—Jaffé,[51] for instance, described subendothelial localization in the splenic vessels of leukemic patients—transmigration has to be dropped and local origin from this part of the vascular wall to be considered. The successful transmission of leukemia in mice by inoculation of a few cells or even of one single immature leukemic cell, as carried out by Furth and Kahn,[52] supports the classification of leukemia as a special type of malignancy and the interpretation of colonization as a special type of metastasis. Here, too, a different possibility of interpretation must not be omitted. Even if leukemia could be definitely established as analogous with malignant neoplasms, it may follow the mechanism of some carcinomas of the skin or of the liver and originate multicentrically.

Local origin is in general more difficult to prove. Definite evidence could be found in a case with negative bone marrow and blood findings combined with myelopoietic foci in liver and spleen. Such a case has not yet been observed, and one may never be observed. It is not likely that the agent which provokes a myelopoietic metaplastic reaction in spleen and liver finds no response in the physiologically myelopoietic bone marrow. Positive findings in the bone marrow, liver and spleen, combined with negative findings in the blood as in aleukemic myelosis, make local origin highly probable, if we can rule out an aleukemic phase of an ordinary leukemia. In most cases of aleukemic myelosis a few immature blood cells can be found in the circulation, and observations in malignant neoplasia prove that very few floating cells are sufficient to give origin to extensive metastases or colonies. The distribution and frequency of the immature blood cells may increase the probability of colonization in single cases. Henschen [53] observed a man of 55 years with spindle cell sarcoma of the thyroid combined with eosinophil leukemia. Roessle, who did the autopsy and analyzed the microscopical findings, considered that the local accumulation of the eosinophile cells, particularly around the necrotic sarcoma deposits, had not only hematogenic but also local origin. He based his conclusion on the distribution of the infiltrating cells, which could not be explained fully by hematogenic invasion, although the patient had 79 per cent eosinophils among 312,000 white blood cells per cmm.

The great difficulty in proving local origin lies in the fact that we can discover the heterotopic myeloid foci of the liver and spleen only at autopsy. At the time when the leukemic patient dies, he generally has reached a progressive phase of his disease with highly positive blood findings which do not permit ruling out colonization definitely. After unsuccessful examination of numerous cases of myelosis, I finally found a case which offers more favorable conditions for proving local origin by presenting not only far-reaching quantitative but also qualitative differences between the immature blood elements of the hepatic and splenic foci and of the blood. There is one more way, as already mentioned, to prove the local origin of extramedullary myeloid foci. It can be accomplished if we can prove the development of the immature blood cells from sessile elements by demonstrating a transitional phase of this transformation *in situ;* that is, cells in the location of sessile elements, which still retain the shape and relations of the sessile cells, but with the nucleus and the protoplasm already showing the characters of immature blood cells. This way is not readily accessible for cells within the interstitial parenchyma, but is available for

endothelial cells, thanks to their characteristic and significant shape and location. Observations of this type have proved the transformation of Kupffer cells into monocytes or into megakaryocytes. The case I observed presented a local transformation of endothelial elements into eosinophile granulocytes.

Report of Case

The patient, a colored woman, 46 years of age, stated that she was well until 3 months before when she began to have digestive disturbance characterized by belching, epigastric fullness, nausea and frequent vomiting. About the same time she had noticed weakness. These symptoms progressed until at the time of examination she could not do her own housework and vomited at least once a day. About 3 weeks before she had noticed abdominal swelling and pain, which was constant, dull, and located in the left upper quadrant. During the preceding 3 days she had vomited practically after every meal.

Physical examination showed the temperature to be 101.8° F.; pulse, 114 per minute; respiration, 28 per minute; blood pressure, 140/90 mm. Hg. The heart and lungs were essentially negative. The abdomen was distended three fingersbreadth above the xiphoid-pubic line. The spleen was enlarged to fill the entire left abdomen and extended into the pelvis. The liver was two fingersbreadth below the right costal margin. In the right side of the pelvis, a rounded, grapefruit-sized mass was palpable. There was tenderness in the left upper quadrant.

Upon *vaginal examination* there was found a firm mass connected to the cervix and extending into both adnexal regions and anteriorly above the symphysis.

The *laboratory findings* were: Hemoglobin, 35 to 36%; red blood cells decreasing from 2,380,000 to 1,970,000; white blood cells decreasing from 196,800 to 141,200; lymphocytes, 2%; myeloblasts, 52%; neutrophile metamyelocytes, 5%; neutrophile myelocytes, 8%; polymorphonuclears, 30%; microcytosis, 2 plus. The bone marrow obtained by sternal puncture showed: 46.6% myeloblasts, 4.6% neutrophile promyelocytes, 1.4% neutrophile myelocytes, 2.2% neutrophile metamyelocytes, 3.4% eosinophile metamyelocytes, 6.2% neutrophile bandforms, 1.8% eosinophile bandforms, 4.2% polymorphonuclear neutrophils, 0.2% polymorphonuclear eosinophils, 22.0% basophils, 4.6% lymphocytes, 0.2% monocytes, 0.2% histiocytes, 0.4% irritation lymphocytes, and 2% normoblasts. The bone marrow was extremely hyperplastic. Maturation of the white cells showed a marked shift to the left, 46% of the cells being myeloblasts. Red cell formation was markedly depressed. A Kahn test was negative. Nonprotein nitrogen in the blood serum was 42 mg. %; creatinin, 1.4 mg. %; uric acid, 6.5 mg. %. The patient received several blood transfusions and ran a continuous septic temperature. Gallop rhythm developed 3 days after admission and she died 1 month after admission.

The *diagnosis* was acute myelogenous leukemia in a phase of acute exacerbation.

Gross Examination

The body (163 cm., 68 Kg.) was that of a well developed, well nourished colored woman. There was moderate chronic pitting edema of both legs. The pupils were contracted, round and equal, the conjunctivae, lips and oral mucosa were pale, as were also the nailbeds. The abdomen was slightly above the level of the chest. There were numerous pale striae over both lower quadrants of the abdomen and over the anterior aspect of both legs.

On opening the *abdominal cavity* the liver was found to extend 20 cm. below the xiphoid process in the midline and 3.5 cm. below the costal margin in the right axillary line. The enormously enlarged spleen extended to the navel and touched the left part of the uterine fundus. The uterus was markedly enlarged, of firm consistency and rendered irregular by several intramural and subserous fibroids. The fundus extended to the navel.

The *heart* weighed 430 gm., and was moderately dilated. The myocardium was pale, brownish and rather soft. The wall of the left ventricle measured 14 mm.; of the right, 3 mm. Both ventricles were filled with firm, pale yellow blood clots and similar clots were found in the large vessels.

Both apices of the *lungs* were free. All lobes were crepitant and on section the surface was light pinkish gray and moist.

The tracheal and bronchial *lymph nodes* were of normal size, soft and slightly anthracotic.

The mucosa of the *esophagus* was pale gray and had low folds. Over the left side of the cardia extended a large ulcer, 2 to 3 mm. deep, measuring 21 by 40 mm. The edges were irregular and sharply demarcated, but not undermined, the smooth gray floor was covered with a thin coat of brownish black, soft hemorrhagic material. The lower pole of the ulcer was 4 mm. below the junction of the gastric and esophageal mucosa. (Fig. 4.)

The *spleen* weighed 2560 gm., the consistency was firm, the capsule slightly thickened, the color dark purple-red, mottled with irregularly shaped, whitish, partially confluent spots. On transverse section it was dark purple, red and brown, mottled with a few paler, grayish areas. In the subcapsular region a few triangular, blackish areas appeared, representing old hemorrhagic infarcts. Where the pale areas reached the surface, the latter was slightly depressed.

The *liver* weighed 3250 gm., being markedly enlarged and firm. The capsule was thin and smooth. The sectioned surface was light brown and distinctly marked by a light periphery and slightly darker center of each lobule and by slightly enlarged periportal fields.

The *kidneys* weighed 325 gm. together and were soft. The capsule stripped with ease leaving a smooth, pale gray surface with distinct markings. On transverse section the cortex measured 9 mm., the pyramids, 12 mm. Their markings were indistinct and both were very pale. The mucosa of the pelvis was smooth and white.

The *uterus* measured 22 by 14 by 7.5 cm., containing several firm, intramural and subserous fibroids, the largest of which had a diameter of 7 cm.

The *bone marrow of the femur* was moderately soft. In part, it was of increased consistency comparable to the consistency of young granulation tissue; in part, soft and gelatinous; the color throughout was light pinkish gray.

The *lymph nodes* were of normal size and consistency. No enlarged or indurated lymph nodes were found.

Microscopical Examination

Liver. In size and shape the hepatic lobules were normal and the lobular structure was distinct. The central veins were slightly dilated and so were, in general, the central parts of the sinusoids, with resulting compression of the central ends of the liver cell trabeculae. Whereas the liver cells in the peripheral part of the trabeculae presented vesicular pale-staining nuclei and protoplasm with distinct plastosomes, the compressed cells had darker staining homogenous protoplasm loaded with brown bile pigment granules. In many acini the paracentral sinusoids were markedly dilated by immature white blood cells, mostly stem cells or myeloblasts with basophile protoplasm. This accumulation of myeloid elements formed small pools extending between the compressed ends of the trabeculae. The portal triads presented a dense infiltration of the stroma, particularly around the portal veins, with some stem cells and numerous myelocytes and metamyelocytes, the majority of which were eosinophils. (Figs. 5 and 6.) Some eosinophile cells were seen in an irregular distribution in the wall of the portal veins. In the immediate vicinity of the bile ducts the eosinophils were not as numerous. The lumina of the vessels in the periportal fields contained few red blood cells, numerous stem cells with basophile protoplasm, promyelocytes, and only a very few eosinophile granulocytes. Whereas the numerous eosinophile cells in the stroma of the periportal fields were mostly of the early immature type—promyelocytes or myelocytes with round or oval, vesicular, pale-staining nuclei—the very few intravascular and intracapillary eosinophils were of the more mature type, mostly metamyelocytes with darker staining, bean-shaped nuclei. Some were even polymorphonuclears, with bisegmented dark nuclei.

Special staining, particularly with Giemsa's stain, showed that many endothelial cells of the sinusoids, while still presenting the normal stellate appearance and typical localization at the Disse space, had developed groups of typical eosinophile granules in their protoplasm. (Figs. 7 and 8.) Some possessed granules only on one side of the nucleus, some on both sides; others had the entire protoplasm filled with the granules. Among the last group, cells were found in which

the spindle-shaped projections of the protoplasm were retracted. In consequence, these cells, the nuclei of which had become more or less round and vesicular, were oval or spherical. Some cells in this phase of transformation were still in touch with the wall at a part of their circumference, other were free-floating in the lumen of the sinusoids. By a series of pictures, the transformation of the sessile endothelial cells, some of which were typically stellate, into free-floating eosinophile promyelocytes could be followed. The transformation was carried through *in situ* and only afterwards were the cells detached. Other endothelial cells were enlarged and their swollen protoplasm contained remnants of phagocytosed red blood corpuscles and of phagocytosed granulocytes. A few only contained small clusters of clumped, faded eosinophile granules, representing the last residues of digested eosinophils. This picture is entirely different from the transforming endothelium and cannot be confused with it. Neither the smaller nor the larger branches of the hepatic vein showed eosinophile cells in or next to the wall. Since liver cell trabeculae are anchored in the walls of the hepatic veins, there is neither an adventitia nor a perivascular stroma present which could contain undifferentiated cells ready for transformation. (Fig. 9.)

Evidently, not only are the external layers of the wall different in the hepatic and portal veins, but the endothelial cells also have different biological activity. In some cases of hemolytic anemia, when the Kupffer cells are loaded with hematogenic iron, the endothelial cells which line the hepatic veins similarly are stuffed with iron pigment, thus proving that they assume reticulo-endothelial functions analogous to those of the Kupffer cells. The endothelium of the intima of the portal veins (not seen in the illustration) remains absolutely free from iron pigment.

Spleen. The microscopical structure of the spleen was indistinct, due to numerous small and large and frequently confluent infarcts of varying ages. The greater part of the parenchyma was thus destroyed. The well preserved remainder presented no partition into follicles and pulp but instead a homogenous tissue consisting mostly of stem cells, myeloblasts, and some myelocytes. Many of these cells had undergone necrosis, as was shown by the homogenous, almost black, partially fragmented, pyknotic nuclei. The trabeculae were thin and indistinct. The stroma contained numerous thin-walled vessels and capillaries. Sinuses were indistinct. The parenchyma presented small groups of eosinophile elements, mostly promyelocytes and myelocytes. Some of these cells had stellate protoplasm with spindle-shaped projections which contained few or no granules. Evidently these cells had

developed from reticular cells of the pulp. In the lumina of the blood vessels was the same abundance of stem cells as in the peripheral blood of other organs and only a very few eosinophile cells, mostly metamyelocytes.

Kidney. The renal glomeruli were normal. The tubular epithelium was high cuboidal with finely granular protoplasm and pale-staining nuclei, some of which had completely faded. There was no myelopoietic tissue. The blood vessels contained the same immature myeloid cells as elsewhere, relatively many more red blood cells and few eosinophils.

Bone Marrow. The bone marrow showed extensive fibrosis. Large areas were occupied by interwoven bundles of collagenous connective tissue interspersed with small clusters of myelogenous cells. The hematopoietic tissue was restricted to about one-third of the space. It consisted of the same immature elements as seen in the foci in the liver, similarly interspersed with a great number of eosinophile promyelocytes and myelocytes. In some areas, the eosinophile cells were increased to about 90 per cent of all cells. Among the fibroblasts in the transitional borderline zones between hematopoietic and fibrotic tissues, spindle-shaped cells with longitudinal vesicular nuclei could be seen which contained eosinophile granules singly and in groups in the protoplasm. The fact that single collagenous fibers crossed the protoplasm of these cells or developed from it proved that we were not dealing with eosinophile granulocytes squeezed and flattened between neighboring fibroblasts or fibrocytes, but with cells which originate from undifferentiated mesenchymatous elements which, instead of developing normally into fibroblasts or into granulocytes, were undergoing a type of mixed or hybrid differentiation. They presented the form and the function of producing interstitial fibers like fibroblasts and granulation like that of eosinophile myelocytes. Transitional pictures made it likely that in the course of further development these cells lose their granulation and join the field of the fibrocytes.

Esophagus. The ulcer of the esophagus showed an almost vertical break in the stratified epithelium. The floor of the ulcer extended 3 to 4 mm. beneath the surface of the epithelium. It was formed by a 0.5 mm. layer of necrotic tissue, which still revealed a dense infiltration with immature white blood cells. This infiltration extended to the muscularis propria and split into small groups which could be found in the septa between the bundles of the circular layer. The cells were of the same types as were found in other organs, with no eosinophils among them. The blood vessels contained, as in the kidney, a high percentage of red blood cells with immature granulocytes and only very few mature eosinophils.

Discussion

Eosinophil leukemia is not frequent compared to neutrophil leukemia. It seems that the frequency corresponds approximately to the physiological relation of eosinophile to neutrophile granulocytes, *i.e.*, about as 1 is to 75. The older literature can be found among the 2000 references which Schwarz [54] abstracted in his almost complete review on eosinophilia published in 1914. The more recent observations are critically reviewed by Forkner,[55] Ringoen [7] and Richter.[56] Forkner analyzed several cases, among them five cases of acute eosinophil leukemia. No definite etiological factor can be given why these cases developed eosinophil and not, as do the great majority, neutrophil leukemia. Constitutional hereditary variations may be considered, but the fact that in some cases changes from neutrophil into eosinophil leukemia and vice versa have been observed, means that not too much emphasis should be placed on constitutional factors.

For local eosinophilia, split proteins, the structure of which is still unknown, have been considered as the incentive by Fiessinger [57] and others. Henschen [53] suggested a good practical classification of the various types of eosinophilia. He distinguished eosinophil chronic myelosis from zoogenic (parasitogenic), anaphylactic, compensatory, neurogenic (parasympathogenic) and constitutional eosinophilia and tried to find a common mechanism for all by explaining them as reaction against still unknown, endogenous or exogenous proteins. His endeavors were contradicted by his collaborator Roessle who, in the discussion, emphasized that it had been impossible to find any common causal factor for the various types of eosinophilia.

Fibrosis of the bone marrow or myelofibrosis has been observed in some cases of leukemia. Reviews have been published by Wolf,[58] and by Mettier and Rusk.[59] The combination with polycythemia has been discussed by Hirsch.[60] The evaluation of some observations and statistics is complicated by the confusion of the terms osteosclerosis and myelofibrosis which signify entirely different changes. Osteosclerosis means concentric increase of the compact layer of the long bones at the expense of the spongiosa, by transformation of parts of the spongiosa into compacta through filling of the interstices between the bone trabecula with solid bone substance. Myelofibrosis means the degenerative replacing of the bone marrow by nonspecific fibrous tissue, analogous to the degenerative replacement by fat tissue in anemia. Osteosclerosis frequently is followed by myelofibrosis; primary myelofibrosis in general is not followed by osteosclerosis. In my case we have to deal with a pure myelofibrosis without evidence of osteosclerosis. The undifferentiated stem cells of the bone marrow which give

origin to red and white blood corpuscles probably are not the "youngest" elements of this tissue. In the reticulum of this mesenchyma, evidently cells are left which represent a still earlier embryonic and less differentiated or specialized phase, cells which may produce blood elements, endothelial elements, or unspecific fibrous tissue. Conditions are similar in the spleen, where, as Klemperer [61] has suggested, mesenchymatous elements are present endowed with the capacity to form blood, interstitial, endothelial, and reticular cells. The differentiation of these cells, predominatingly or exclusively, into interstitial unspecific elements, causes fibrosis of the spleen as in splenomegaly with hepatic cirrhosis. In my case we see that these elements still produce myeloid and erythropoietic stem cells, but to a great extent they change into fibroblasts, which form nonspecific and even hyaline degenerating connective tissue, thus restricting the hematopoietic tissue.

Ulcers of the intestines developing from leukemic infiltrations have been observed in many cases. One of the earliest reports on leukemia, a case analyzed by Virchow [62] in *Die krankhaften Geschwülste,* includes the description of leukemic ulcers in the upper ileum. Later, leukemic intestinal ulcerations developing usually from leukemic infiltrations have been described by Eichhorst [63] (case with fatal hemorrhage), Schultze,[64] Herxheimer,[65] Askanazy,[49] Wells and Mayer [66] (who coined the term pseudoleukemia gastrointestinalis for diffuse and nodular gastrointestinal leukemic infiltration with secondary ulceration) and v. Müllern and Grossmann.[67] Boikan [68] published in 1931 a review of the literature with some cases of his own. Following the conception of Jaffé,[69] he concluded from the similarity of the intestinal ulcerations in true leukemia and in aleukemic leukemia that these two diseases are identical. Recently Jones [70] described two cases, one with perforation of a leukemic ulcer at the ileocecal junction. Ebstein,[71] Mager [72] and Barnick [73] have observed analogous ulcerative leukemic changes in the pharynx and larynx, and Askanazy [49] described the histological picture of corresponding changes of the gingiva. An ulcer of the esophagus has not yet been described, as far as I could check the literature.

This case is one of chronic myelogenous leukemia, evidently before the outbreak of an eosinophilic phase. The immature eosinophils are already in the tissues but not yet in the circulation in any considerable number. For the eosinophils, the aleukemic stage is just on the verge of changing into a leukemic phase. The production of the eosinophils in the bone marrow is not different from that found in cases of eosinophil leukemia. In the liver they develop from two sources: from the

endothelial cells of the sinusoids and from elements in the adventitia and in the stroma next to the vascular wall. The transformation of the sinus endothelium, which can be proved by the presence of transitional phases, gives full evidence of local origin of this type of granulocytes. For the eosinophils in the periportal fields, local origin is most probable. There is no evidence that the eosinophils which have developed from Kupffer cells immigrate in the liver parenchyma and settle about the periportal triads. Such wandering of primarily sessile vascular endothelial cells, marked by the phagocytosis of injected India ink, through the connective tissue to distant points has been observed by Herzog [50] in the frog's tongue. In my case there is no relation between the eosinophils of endothelial origin and those of perivascular origin, except that both enter the blood circulation. Colonization cannot be ruled out since there are eosinophils in the blood. But these circulating eosinophils on the average are of much higher maturity than the perivascular eosinophils in the liver triads, and a local rejuvenation of blood cells after colonization is most unlikely. Sections of the white, firm blood clot found in the left ventricle at autopsy gave a good impression of the smaller proportion of eosinophils of relatively higher maturity in the blood, as compared to those in the liver tissue. Whether in the periportal triads they developed from the adventitia and from elements of the surrounding stroma, or from the adventitia only and secondarily swarmed out in the neighborhood, cannot be decided by the analysis of the slides. If there was a simultaneous development in the adventitia and in the surrounding stroma, we have to suppose that the cellular elements in the stroma which are prone to this transformation are the more numerous the nearer to the adventitia the tissue is located. For the sinus endothelial cells, this case shows that in addition to the potency to form monocytes physiologically (Aschoff [74]) and in monocytic leukemia (Jaffé [33]), and megakaryocytes in atypical leukemia (Downey, Palmer and Powell [32]), they also have the potency to change into eosinophile granulocytes.

Summary

Two explanations have to be considered for the development of extramedullary myelopoiesis. The first is the assumption of local origin. This theory meets with greater probability when the organs in question are those normally concerned with hematopoiesis in fetal life, as the liver and spleen. In the anemias, the presence of erythropoietic tissue in these organs may be interpreted as a compensatory revival of an earlier function which physiologically is not carried on in extra-uterine life. A subgroup of local myelopoiesis which finds

good support in many animal experiments concerns the transformation of vascular endothelium (Dieckmann [14]), of perivascular adventitia cells (Bloom,[40] Herzog [50]), or of undifferentiated mesenchymatous elements mostly located in the vicinity of blood vessels (Jaffé,[69, 75] Lang [10, 23]) into undifferentiated lymphoid cells or hemocytoblasts, after irritation or stimulation by injection of dead bacteria, as *Bacillus proteus* or *B. coli;* of poisons, as saprotoxin, or pyrogallol; or after inoculation with transplantable tumors. The second theory traces the extramedullary foci of myeloid tissue back to hematogenous immigration of immature bone marrow cells which enter the circulation. This process, which is analogous to the metastasizing of malignant tumor cells by the blood stream, has been called by some authors *colonization,* a term coined instead of using metastasis in order to avoid an anticipated analogy of leukemic blood cells with malignant neoplastic cells.

For the extramedullary myelopoiesis in human leukemia, the question, as Boyd [48] points out, has not yet been decided, whether we have to deal with autochthonous local origin or with colonization. A high percentage of cells in the blood and in the bone marrow, small perivascular foci, and the histological picture of numerous immature bone marrow elements in process of migration through vascular walls, make colonization probable. Local origin is much more difficult to prove. Two ways are open. The first of these is to find evidence of local transformation of mesenchymatous cells, particularly of cells of the reticulo-endothelial system, into immature blood cells, that is, metaplasia *in situ,* by which these cells change into blood cells. Such observations are in general very difficult when reticular elements are concerned, but easy and obvious as far as endothelial cells are concerned, which by shape and localization are well characterized and easily recognizable. Bloom [1] succeeded in animal experiments in provoking transformation of Kupffer cells into megakaryocytes. The analogous change was observed in a case of atypical leukemia by Downey, Palmer and Powell.[32] Jaffé [33] presented in one of his conferences a case of monocytic leukemia with positive oxidase reaction of the Kupffer cells. I have found similar changes in a case of chronic myelogenous leukemia in an acute monocytic phase. The liver showed the same change of the Kupffer cells, which, after transformation into round or oval cells, entered the circulation as large oxidase-positive monocytes.

The second way is to find a case which shows an abundance of the immature blood elements in the bone marrow and in well developed extramedullary foci, with the circulating blood free from such cells. Quantitative differences between the incidence of immature blood cells

in the blood and in the tissue do not prove much, since even a few intravascular cells may give origin to extensive metastases as we observe in malignant tumors. Only qualitative differences are convincing, that is, absence of the immature elements in the blood, which are present in the tissues. Application of this criterion meets with the difficulty that generally the blood is highly abnormal in the phase in which a patient with leukemia comes to autopsy. Only if, by chance, a patient dies at a time when the tissues contain great numbers of immature blood elements which not only are absent in the circulation at the time of autopsy but, as proved by the history, never were present (to avoid confusion with aleukemic leukemia or an aleukemic phase of a common leukemia), can it be applied. Only then can local origin of the extramedullary foci be established. After unsuccessful examination of numerous cases of acute and chronic lymphatic and myelogenous leukemia, a case of a colored woman, 46 years old, with chronic myelogenous leukemia was found, who expired at a time when the neutrophil leukemia was on the verge of changing into an eosinophil leukemia. Whereas the bone marrow and the liver at autopsy contained huge numbers of eosinophile promyelocytes and myelocytes, only the last blood examination a short time before death showed a low percentage of eosinophile metamyelocytes and polymorphonuclears. Slides of the liver gave evidence of a gradual transformation of Kupffer cells into eosinophile myelocytes by developing eosinophile granules and retraction of the stellate protoplasmic projections. After this metaplastic change, the cells entered the blood of the sinusoids as eosinophile granulocytes.

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[*Illustrations follow*]

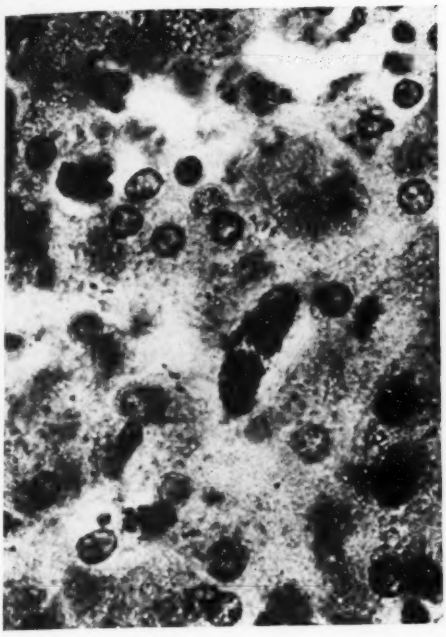
DESCRIPTION OF PLATES

PLATE 99

FIG. 1. From a patient with monocytic leukemia. Liver with oxidase-positive granules in the Kupffer cells. A spindle-shaped Kupffer cell can be seen in the center, the protoplasm of which is stuffed with oxidase-positive granules. The lumen of the sinusoid at the top contains several oxidase-positive mononuclear cells. Oxidase reaction of Goodpasture. × 480.

FIG. 2. From the same patient as Figure 1. Kupffer cell with numerous oxidase-positive granules, surrounded by five liver cells in the corner of a sinusoid.

FIG. 3. Periportal cirrhosis and hemochromatosis in a white man, 59 years of age. Specimen obtained from the liver for biopsy. The Kupffer cells, particularly in the upper right portion of the field, are full of iron pigment, and so, also, are the endothelial cells of the small branch of the hepatic vein at the left. Turnbull's-blue stain. × 50.



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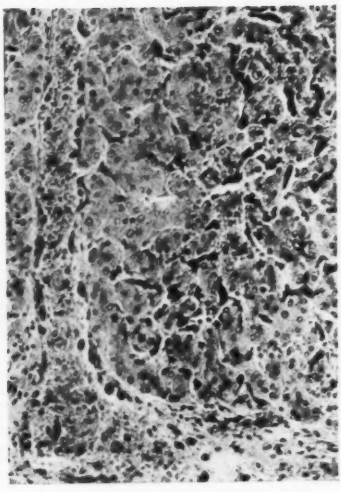

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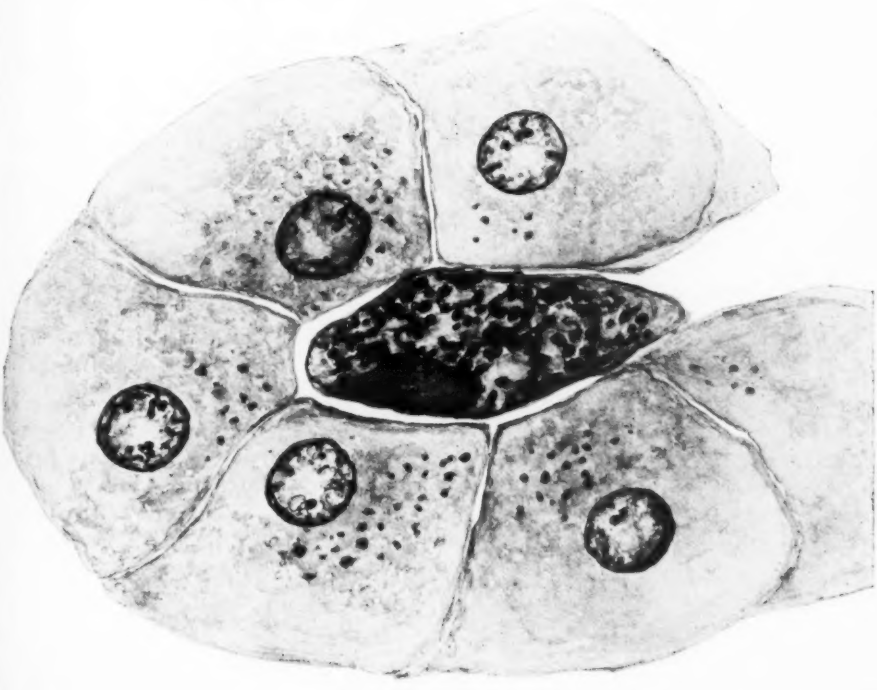


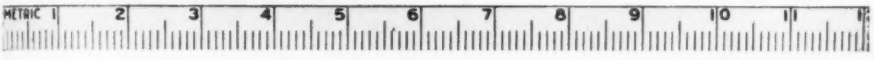
2

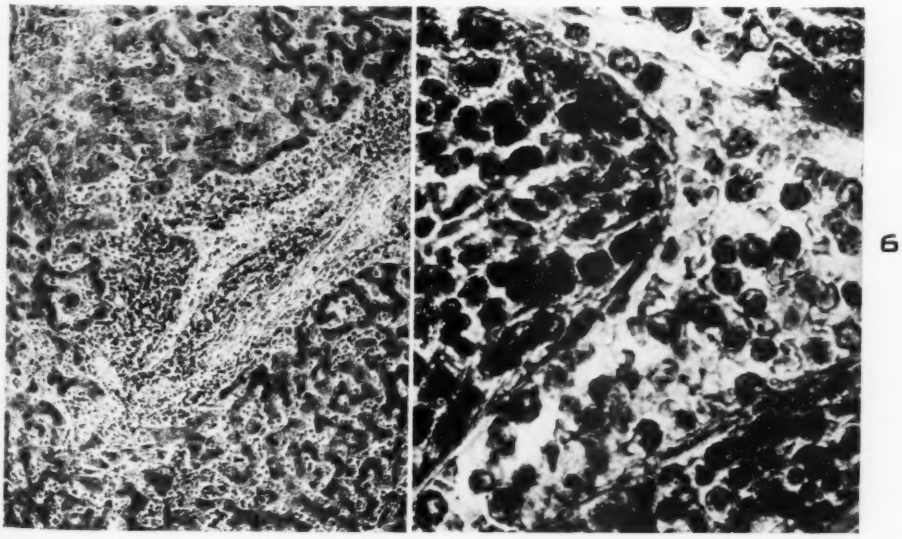
PLATE 100

FIG. 4. From a patient with myelogenous eosinophil leukemia. Ulcer of the esophagus at the cardia.

FIG. 5. Myelogenous eosinophil leukemia. The stroma around a branch of the portal vein is infiltrated with eosinophile myelocytes. The lumen of the vein contains only a very few mature eosinophils. × 70.

FIG. 6. The bifurcation of the vein shown in Figure 5. × 355.





Schiller

Local Myelopoiesis in Myeloid Leukemia

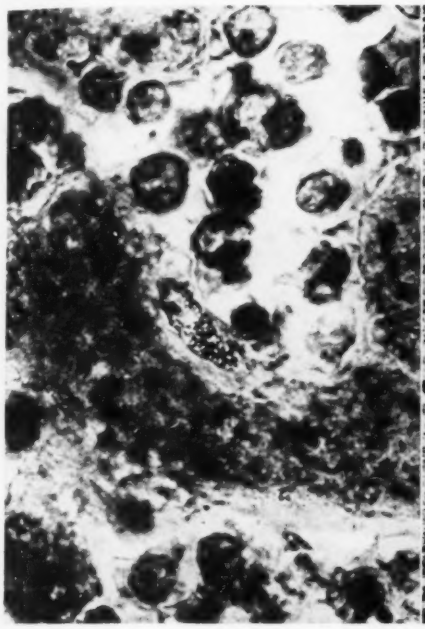
Plate 101

Figures 7, 8 and 9 were made from sections from the same case of myelogenous eosinophil leukemia as the preceding three illustrations.

Fig. 7. One Kupffer cell in the sinusoid is loaded with eosinophile granules. × 800.

Fig. 8. Three Kupffer cells have developed eosinophile granules. There is swelling of the protoplasm as a result of the granulation. In the upper part of the lumen there is a large, square, free-floating phagocyte, the protoplasm of which contains palely staining remnants of partially digested red blood cells.

Fig. 9. A small branch of the hepatic vein at the point of union with a larger trunk. There are no eosinophils along the walls. × 80.



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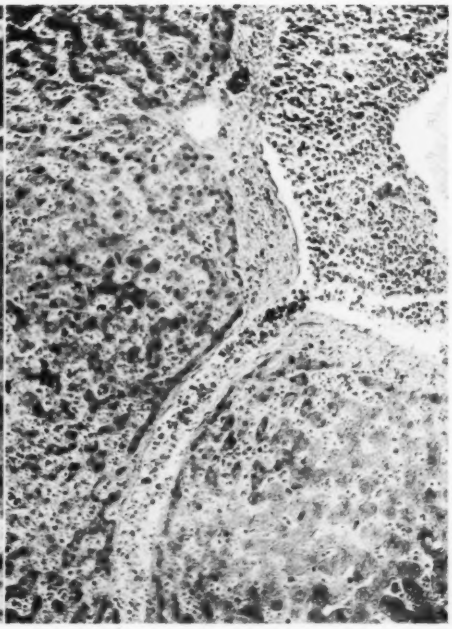

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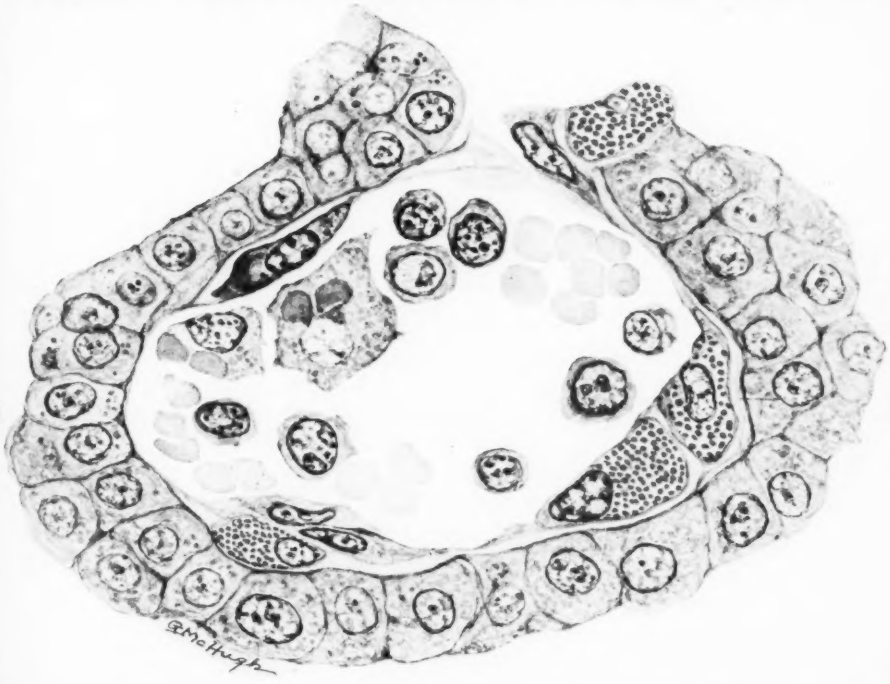

8

THE EFFECTS OF PARATHYROID HORMONE AND CALCIUM GLUCONATE ON THE SKELETAL TISSUES OF MICE *

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The results of experiments on the effect of parathyroid hormone on the skeleton are not in full agreement. Cartilaginous overgrowth, stunting, or lack of any effect on body growth has been reported. On the other hand, increased resorption as well as increased formation of bone has been noted. The discrepancies in the findings seem to be due to several factors, such as species and age of the animal, the dose of the hormone, and the calcium and phosphorus intake. It is still being discussed [1] whether the temporary rise in the serum calcium is a primary effect of parathyroid hormone, or whether it is secondary due to an increased phosphorus excretion.[2] Administration of calcium gluconate likewise raises the serum calcium, although to a lesser degree and for a shorter period than parathyroid hormone.[3-5]

In continuation of former investigations on the effects of hormones on cartilage and bone,[6] we thought it of interest to study whether increased serum calcium on the one hand, and parathyroid hormone on the other, affect skeletal development and ageing.

MATERIAL AND METHODS

Seventy-six male mice of the closely inbred strains C57 and CBA were used. The animals were kept on a standard diet of Purina chow with water available at all times. One group consisted of 38 growing mice, 4 to 9 weeks old at the beginning of the experiment. A second group consisted of 38 adult mice 12 to 14 months old at the beginning of the treatment. Further details are given in Table I.

The following three series of experiments were carried out:

Series I (28 Mice). Fourteen growing and 14 adult animals were injected intraperitoneally with parathyroid hormone.† Two animals from each group were treated daily for 1, 2, or 4 consecutive days, and 2 animals were injected three times weekly for 1, 2, 4, or 8 weeks.

* These experiments were conducted in the Laboratory of Research Pathology, Washington University, School of Medicine, St. Louis, Mo.

The investigation was carried out by the aid of grants from the International Cancer Research Foundation, from the Jane Coffin Childs Memorial Fund for Medical Research, and from the Committee on Research in Endocrinology of the National Research Council, given to Dr. Leo Loeb; and by the Albion O. Bernstein Fellowship in Pathology, New York University, College of Medicine.

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† Parathormone, Eli Lilly and Co., Indianapolis, Ind.

TABLE I

Mean Initial and Final Weights and Deviations from These Means in Pairs of Treated Mice

	Growing mice				Adult mice		
	Duration of experiment	Age at beginning of experiment	Initial weights gm.	Final weights gm.	Age at beginning of experiment	Initial weights gm.	Final weights gm.
A. Mice receiving parathyroid hormone	1 day	6 weeks	15.4 ± 1.0	13.0 ± 0.2	14 months	21.5 ± 0.5	20.5 ± 0.5
	2 days	6 weeks	16.1 ± 1.0	13.9 ± 0.3	14 months	23.2 ± 0.2	19.8 ± 0.0
	4 days	6½ weeks	18.0 ± 2.0	19.4 ± 2.3	13 months	28.1 ± 1.4	29.5 ± 2.0
	1 week	4½ weeks	10.9 ± 0.5	15.0 ± 0.6	12 months	27.3 ± 0.9	28.0 ± 0.2
	2 weeks	5½ weeks	12.3 ± 1.4	22.2 ± 0.2	14 months	23.8 ± 1.7	27.0 ± 2.0
	4 weeks	6 weeks	17.0 ± 0.4	23.5 ± 1.6	12 months	27.9 ± 1.2	27.9 ± 1.2
	8 weeks	5½ weeks	13.2 ± 0.6	23.9 ± 1.0	12 months	28.5 ± 0.7	28.5 ± 0.7
B. Mice receiving calcium gluconate	1 day	4 weeks	9.3 ± 0.5	9.5 ± 0.5	13 months	27.7 ± 2.3	28.0 ± 1.5
	2 days	5 weeks	12.2 ± 0.2	11.4 ± 0.2	13 months	26.4 ± 0.6	25.5 ± 0.2
	4 days	4 weeks	10.3 ± 0.0	9.9 ± 0.0	12 months	19.6 ± 0.0	22.8 ± 0.0
	1 week	6½ weeks	16.5 ± 0.8	16.4 ± 0.7	14 months	26.9 ± 4.0	25.1 ± 5.2
	2 weeks	5 weeks	11.9 ± 0.0	16.6 ± 0.0	12 months	26.8 ± 4.5	28.2 ± 3.5
	4 weeks	8 weeks	20.5 ± 0.0	24.7 ± 0.0	12 months	30.3 ± 0.4	30.2 ± 0.4
	8 weeks	5½ weeks	12.8 ± 1.0	24.7 ± 1.5	12 months	26.4 ± 0.0	28.8 ± 0.3
C. Mice receiving parathyroid hormone and calcium gluconate	1 week	4 weeks	10.1 ± 0.0	10.4 ± 0.3	12 months	26.3 ± 2.5	25.7 ± 3.0
	2 weeks	5½ weeks	14.7 ± 0.2	20.5 ± 0.3	12 months	23.6 ± 0.0	25.2 ± 0.0
	4 weeks	9 weeks	22.4 ± 0.2	23.5 ± 0.7	13 months	28.5 ± 2.1	25.8 ± 3.6
	8 weeks	6 weeks	15.0 ± 1.1	25.5 ± 1.3	12 months	25.2 ± 1.3	29.2 ± 1.0

Growing animals received 4 units, adult animals 7 units of the hormone at each injection.

Series II (*28 Mice*). Fourteen growing and 14 adult animals received intraperitoneal injections of 0.25 cc. of a 10 per cent solution of calcium gluconate.* Two animals from each group were treated daily for 1, 2, or 4 consecutive days, and 2 animals from each group were injected three times weekly for 1, 2, 4, or 8 weeks.

Series III (*20 Mice*). Ten growing and 10 adult animals received a combined administration of parathyroid hormone and calcium gluconate for 4 consecutive days, 1, 2, or 8 weeks. The dose administered corresponded to that given in series I and II.

As far as possible, litter mates were used. Additional mice of the same strains, sex and age served as normal controls. Sections of tibia and femur were prepared as described in previous investigations.[7] The epiphyseal disk at the upper tibia was selected as the area where any changes could be best studied.

A. EFFECTS OF PARATHYROID HORMONE

1. Growing Animals

(a) Gross Observations

The treated animals lost 10 to 20 per cent of their initial weights after the first two injections (Table I, section A). The initial weights were regained after the fourth injection. Subsequently, the weight increase was rapid and surpassed that of noninjected animals.[8] After 1 and 2 months of treatment, the weights equalled those of noninjected mice.

(b) Histologic Examination

(*1*) *Epiphyseal Disk.* After one injection of 4 units of the hormone, the zone of endochondral ossification showed regular structure in the cartilaginous cell columns. The cartilage rows were separated from one another by thin strips of chondromucoid ground substance. The total number of cells in a single cartilage row was 10, as is normal for this age. However, the various cells were larger than the corresponding cells in untreated animals. The nonoriented cartilage cells were rounded off; their conversion into columnar cartilage cells was accelerated. The latter cells exhibited marked proliferation by mitosis. The onset of hypertrophic changes took place farther proximally than in control mice. The replacement of the hypertrophic cartilage cells by bone was increased (Figs. 1 and 2).

After two injections, the columnar cells in a single column had decreased to 6 or 7 (instead of 10), and the hypertrophic cells to 2 or 3

* Abbott Laboratories, North Chicago, Ill.

(instead of 4, as is normal). Simultaneously, more calcium than usual had been deposited in cartilage cells and matrix. The nonoriented cartilage cells had assumed a resting condition. The proliferation and hypertrophy of the cartilage were decreased. The ossification of the densely calcified cartilage was more accentuated than after one injection.

After 4 days' treatment, the growth zone was heavily calcified (Fig. 3). The proliferation of the columnar cartilage was greatly diminished or at a standstill, but the replacement of cartilage by bone was increased.

At later stages, the cartilage underwent marked regression. Degenerated cartilage rows appeared in the epiphyseal plate of mice 7 or 8 weeks old injected for 2 weeks. These changes were not found in healthy untreated animals of this strain before the end of the fourth month of life.

After 1 or 2 months of treatment, the epiphyseal disk was distinctly narrowed. Numerous thick, amorphous, sometimes ossified plugs had replaced the degenerated cartilage rows. In addition, increased resorptive processes had begun to dissolve the amorphous or bony plugs, and perforations of the epiphyseal plate were noted (Fig. 4). Thus, in 10 weeks old mice of strain C57, injected for 4 weeks, skeletal development and ageing had reached a degree seen in healthy noninjected mice of this strain only toward the end of the first year of life.

(*2*) *Subepiphyseal Layer.* In untreated animals, the subchondral zone consists of a loose, vascular connective tissue, and of bony spicules separated from one another by cellular marrow. After one injection, osteoblasts proliferated mitotically and filled the intertrabecular spaces (Fig. 2). Farther distally, along the pre-existing bony spicules, the mitotic proliferation of osteoblasts was likewise stimulated (Fig. 5). These changes were further accentuated after two injections. The conversion of osteoblasts into osteocytes was increased; the trabeculae were thickened, elongated, more numerous, and connected with one another by bony links.

After 4 days of injections, the spicules were numerous and densely calcified, and a transverse osseous plate was being formed underneath the epiphyseal cartilage.

After 1 or 2 weeks, cellular and vascular resorption predominated over the formation of bone, and the excess bone present at the earlier stages disappeared. After 1 or 2 months of treatment, the metaphyseal spicules in the center had been dissolved, whereas at the periphery there were still numerous thickened trabeculae. The bone marrow had not undergone fibrotic change.

(3) *Joint.* During the first 2 days of treatment, the articular cartilage cells were hyperplastic and hypertrophic. Later, the hyperplasia ceased, and the increased hypertrophy was followed by intensified ossification. After 2 weeks of injections and later, the cartilaginous matrix had increased in amount and density.

(4) *Shaft.* Subsequent to the first, second and fourth injections, the compacta was thick and dense. The spindle cells at the inner and outer surfaces of the shaft proliferated mitotically. Many of these cells had been converted into large osteoblasts lying close together (Figs. 6 and 7). After continuation of the treatment for 1 or 2 weeks, however, the proportion between spindle cells and osteoblasts was changed in favor of the spindle cells. After 1 month of treatment, the inner surface of the shaft was again lined by a single layer of endosteal cells as is normal (Fig. 8).

II. Adult Animals

(a) Gross Observations

After administration of 7 units of the hormone for 1 or 2 days, the animals lost on the average 10 to 12 per cent weight (Table I, section A). Subsequently, there was a steady weight increase that sometimes surpassed that of control mice.

(b) Histologic Examination

(1) *Epiphyseal Disk.* In untreated old mice, the epiphyseal plate consists of calcified, hyalinized, or ossified cartilage, in some places perforated by bone marrow.

During the first 2 weeks of injections, the ossification of the calcified and hyalinized cartilage was farther advanced than in untreated animals. After 1 or 2 months of treatment, a large part of the strongly calcified cartilage was ossified.

(2) *Subepiphyseal Layer.* In untreated mice, the metaphyseal bone undergoes increasing resorption with advancing age. Following treatment from 1 to 4 days, the transverse osseous lamella underneath the epiphyseal cartilage was thin; in some areas, calcified cartilage was in direct contact with the bone marrow. Simultaneously, an increased number of osteoblasts had been formed underneath the thinned osseous plate and on the surface of such trabeculae as were present. At later stages of the experiment, even more bone had been laid down. Thus, transverse trabecular bony structures of considerable thickness appeared in the metaphysis (Figs. 9 and 10). There was no increase in the amount of connective tissue in the diaphyseal marrow.

(3) *Joint.* Administration of parathyroid hormone did not affect the

incidence and severity of the age changes taking place in the articular cartilage in normal old mice.[9]

(*4*) *Shaft.* No changes were observed under the influence of the hormone.

B. EFFECTS OF CALCIUM GLUCONATE

1. Growing Animals

(a) Gross Observations

The treated animals did not gain, or even lost weight, during the first week of the injections. Subsequently, the weights increased, and after 1 or 2 months they did not differ from those of the control animals (Table I, section B).

(b) Histologic Examination

(*1*) *Epiphyseal Disk.* After one injection, but more pronouncedly after two or four injections, the zone of endochondral ossification was narrower than usual. The cell count was 6 or 7 (normal 10) columnar, and 1 or 2 (normal 4) hypertrophic cells in a single cartilage row in animals 5 weeks old. The nonoriented cartilage cells were resting. The columnar cartilage cells were flatter than ordinarily; their proliferation was decreased, mitoses being scarce or lacking. The hypertrophic cartilage cells were also decreased in size and heavily calcified. The largest cells present had the size of large columnar cells of untreated mice.

After 1 or 2 weeks of treatment, the columnar cartilage was less atrophic and its proliferation was less inhibited than at the earlier stages. Larger hypertrophic cartilage cells were found, and their replacement by bone had made good progress. After 1 or 2 months of injections, the height of the growth zone did not differ from that of the untreated animals. However, the structural age of the epiphyseal cartilage was advanced over the normal. Hyalinization and calcification were marked, and amorphous plugs of disintegrated cartilage appeared in the epiphyseal disk at the upper tibia in mice 12 weeks old (Fig. 11). This condition is not observed in untreated healthy mice of this strain and age. The younger the animal at the beginning of the injections, the more readily it responded to the treatment.

(*2*) *Subepiphyseal Layer.* After 1, 2, or 4 injections, the subchondral zone was poorly vascularized. The bony spicules were well formed and strongly calcified; they consisted of compact osteocytes and dense ground substance. The mitotic proliferation of osteoblasts was not increased. After 1 or 2 weeks of treatment, the vascularization of the subepiphyseal zone had increased; intensified resorption had led to a shortening of the trabeculae which in their proximal parts were

connected by transverse osseous links. At later experimental stages, a bony lamella underneath the epiphyseal cartilage had formed in mice of strain C57 13 weeks old. In untreated animals of this strain, a corresponding condition is not seen before the age of 4 or 5 months.

(3) *Joint.* The articular cartilage showed no significant changes throughout the experiments.

(4) *Shaft.* During the first week of injections, the compacta was heavily calcified and denser than usual. With increasing duration of the experiment, the bony cortex assumed a normal firmness.

II. Adult Animals

(a) Gross Observations

No influence on the weights of the animals was noted (Table I, section B).

(b) Histologic Examination

(1) *Epiphyseal Disk.* At no experimental stage was there any evidence of an effect of calcium gluconate on the inactive cartilage cells.

(2) *Subepiphyseal Layer.* During the first 2 weeks of treatment, the transverse subchondral bony plate was thin. After 1 or 2 months, osteocytes in increased number were laid down along the transverse osseous plate.

(3) *Joint.* The incidence and severity of the age changes found in the articular cartilage did not differ from those noted in untreated animals.

(4) *Shaft.* No deviation from the normal condition could be detected.

C. EFFECTS OF COMBINED ADMINISTRATION OF PARATHYROID HORMONE AND CALCIUM GLUCONATE

I. Growing Animals

(a) Gross Observations

During the first week of treatment, the weights remained stationary. Subsequently, the animals gained weight steadily, and finally the weights equalled those of the controls (Table I, section C).

(b) Histologic Examination

(1) *Epiphyseal Disk.* After 4 days of treatment (8 units of parathyroid hormone and 0.5 cc. of calcium gluconate) the growth zone was narrowed.

The nonoriented cartilage cells were in a resting condition. Proliferation and hypertrophy of the columnar cartilage were decreased; calcification and ossification, however, were intensified as compared with the normal. After 1 week of injections (12 units of parathyroid hor-

mone and 0.75 cc. of calcium gluconate) the epiphyseal disk was narrower than at the earlier stage. In a single cartilage row 6 or 7 (instead of 10) columnar and 2 or 3 (instead of 4) hypertrophic cartilage cells were counted. Calcification and ossification of the cartilage were marked. Subsequently, regression and ossification of the cartilage had increased still further, and thick bony plugs had replaced cartilage rows. These changes did not make progress during the second month of treatment. Resorptive processes were less marked than after administration of parathyroid hormone only. In one 3 months' old mouse, advanced resorption had produced small perforations of the epiphyseal disk. In the three remaining mice, 12 to 15 weeks old, and injected for 1 or 2 months, the structural age of the epiphyseal cartilage was comparable to that of noninjected mice 6 to 8 months of age.

(2) *Subepiphyseal Layer.* After 4 days of treatment, numerous mitotically proliferating osteoblasts had produced an osteogenic tissue. The trabeculae were thicker than normal, and thicker than after injections with parathyroid hormone alone for the same time. After 1 week of injections, intense resorption had begun to remove the bony spicules in the central portions of the metaphysis; however, the deposition of bone in close approximation to the epiphyseal cartilage was still increased, and osseous links between the spicules appeared. With longer duration of the experiment, both formation and resorption of bone remained accentuated. The resorption of bone was less pronounced than after administration of parathyroid hormone, but more marked than after injections of calcium gluconate alone.

(3) *Joint.* During the early experimental stages, ossification of the articular cartilage was intensified; later, resorptive processes predominated. After 2 months of injections, the condition was not different from the normal.

(4) *Shaft.* During the first 2 weeks of treatment, the compacta was thicker and denser than usual. Later, intensified resorption had led to the usual appearance of the cortex.

II. Adult Animals

(a) Gross Observations

Some animals showed stationary weights, others lost weight up to 15 per cent, still others gained weight (Table I, section C).

(b) Histologic Examination

(1) *Epiphyseal Disk.* Throughout the experiments, remnants of markedly calcified, hyalinized, or ossified cartilage were present. The degree of perforations of the disk by bone marrow was not advanced over the normal.

(*2*) *Subepiphyseal Layer.* During the first 2 weeks of treatment, the subepiphyseal bony lamella was thin. After 1 or 2 months of injections, production of bone was prominent. Osseous tissue had been deposited in the corners between the metaphyseal part of the shaft and the heavily calcified remnants of epiphyseal cartilage. An irregular bony network appeared parallel to and underneath the pre-existing transverse osseous plate, and one or several thick bony bars had been laid down in the metaphysis (Fig. 12). This excessive bone formation had inhibited the normal progress of epiphyseo-diaphyseal union.

(*3*) *Joint.* The injections did not affect the incidence nor the severity of the arthropathic age changes found in untreated old mice.

(*4*) *Shaft.* The compacta was thin at the earlier stages of the experiments, as is normal for old mice. After 1 or 2 months of treatment, the cortex consisted of more and denser bone than ordinarily.

Discussion

In immature mice, parathyroid hormone intensifies hypertrophy and ossification, but does not stimulate the proliferation of the epiphyseal cartilage. It increases the mitotic proliferation of osteoblasts and thus increases bone formation. Subsequently, increased regressive and resorptive processes cause a premature onset of perforations of the growth zones and a removal of the excessive amounts of bone. Parathyroid hormone thus promotes the ageing of the epiphyseal cartilage. In old mice, parathyroid hormone increases the calcification of the inactive epiphyseal cartilage and intensifies deposition of bone in the metaphysis.

In growing mice, calcium gluconate promotes temporarily calcification and ossification, but inhibits proliferation, hypertrophy and resorption of the epiphyseal cartilage. It increases the density of the bone, but does not stimulate the mitotic proliferation of osteoblasts. Subsequently, proliferation and hypertrophy of the cartilage are resumed, and regressive and resorptive processes are intensified as compared with the earlier stages. Calcium gluconate thus likewise accelerates skeletal ageing, but to a far less degree and for a shorter length of time than parathyroid hormone. In old mice, calcium gluconate does not affect the inactive epiphyseal cartilage. However, it decreases the resorption of bone. Consequently, in the metaphysis and in the shaft more bone is present than usual.

In growing mice the combined administration of both substances does not intensify the accelerated ageing of the epiphyseal cartilage caused by parathyroid hormone alone. However, the resorption of cartilage and bone is less marked than after injections of parathyroid

hormone only, and more bone is present than after administration of either substance alone. In old mice the combined treatment produces considerable amounts of new bone, particularly in the metaphysis.

Table II summarizes the histologic findings in a schematic way.

The ageing effect that parathyroid hormone exerted on the epiphyseal cartilage of growing mice can be correlated with the gross observations of stunting made in growing rats,[10, 11] dogs [12] and axolotl [13] under the influence of this hormone. Parathyroid hormone not only accelerates the ageing of the epiphyseal cartilage, but may also stimulate the formation of bone. The intensified calcification of the epiphyseal cartilage may by itself promote the production of bone. Moreover, the increased deposition of bone may be the consequence of the stimulation of the connective tissue cells. If, as in our mice, sufficient calcium is available, excessive amounts of new bone are laid down by the increased number of osteoblasts. This osteoblastic proliferation subsequent to the administration of parathyroid hormone is apparently independent of the elevated serum calcium, since it did not occur following injections of calcium only. Increased production of bone under the influence of parathyroid hormone has been reported in growing rats by Bauer, Aub and Albright,[14] Shelling, Asher and Jackson [15] and by Selye,[16] while Burrows [11] found increased formation of bone subsequent to a stage of increased resorption.

In growing mice the increased deposition of bone was followed by increased resorption. This reversal led gradually to a normal structure of the diaphysis; but there was no fibrosis of the bone marrow nor any other changes suggestive of fibrous osteitis, as reported in rats, guinea-pigs, rabbits and dogs after administration of parathyroid hormone. Recent biochemical investigations on the phosphatase content in the diaphysis of rats have demonstrated a certain parallelism between histologic and biochemical changes. Roche and Filippi [17] and Williams and Watson [18] injected rats with parathyroid hormone in doses comparable to those we used in mice. They found the phosphatase in the diaphysis increased during the early stages of the experiments, decreased during later stages, and finally back to normal values.

From our experiments no conclusions can be drawn in respect to whether the dose of the hormone might influence the histologic reaction. The data recorded in the literature are not in agreement. Shelling, Asher and Jackson [15] obtained increased formation of bone when low doses of parathyroid hormone had been administered to rats, but increased resorption of bone when high doses had been injected. Jaffe, Bodansky and Blair,[19] on the other hand, observed increased resorption of bone, irrespective of the dose. That very high toxic doses caused cell

TABLE II

Scheme Demonstrating the Main Effects of Parathyroid Hormone, of Calcium Gluconate and of Both Substances on Cartilage and Bone

		Cartilage		Bone		Onset of epiphyseo-diaphyseal union
		Proliferation	Calcification	Formation	Resorption	
A. Conditions in growing mice	Parathyroid hormone	Decreased	Greatly increased	Much increased; strong osteoblastic proliferation	At later stages increased	Accelerated
	Calcium gluconate	Temporarily inhibited	Somewhat increased	Somewhat increased; no osteoblastic proliferation	First decreased; at later stages slightly increased	Unaffected
	Parathyroid hormone and calcium gluconate	Somewhat decreased	Increased	Greatly increased; some osteoblastic proliferation	At later stages somewhat increased	Somewhat accelerated
B. Conditions in adult mice	Parathyroid hormone		Somewhat increased	Increased	Unaffected	
	Calcium gluconate		Unaffected	Slightly increased	Somewhat decreased	
	Parathyroid hormone and calcium gluconate		Strong	Greatly increased	Slightly decreased	

death (necrosis of osteoblasts),[20] rather than growth of tissue, is not surprising. However, it is doubtful whether these changes are specific effects of the hormone.

The response to parathyroid hormone depends also on the age of the animal at the beginning of treatment.[21] In adult mice, the inactive epiphyseal cartilage responded far less to the administration of parathyroid hormone than the growing cartilage of young animals. Moreover, bone formation was intensified only at later stages of the experiment. These findings represent further evidence for the observations reported previously [21] that longer administration and higher doses of a hormone are required in order to elicit a reaction in older animals. The age factor apparently plays a rôle also in determining the reaction in other species. Old guinea-pigs were found to be far less responsive to the parathyroid hormone than growing animals.[23] Pugsley and Selye [24] observed in adult rats, production of bone preceded by intensified resorption of bone, whereas in suckling rats the first reaction to the parathyroid hormone consisted of increased formation of bone.

In growing mice, calcium gluconate likewise accelerated the ageing of the epiphyseal cartilage and favored formation of bone. These findings are in agreement with gross and biochemical observations of increased production of bone in rats,[25, 26] guinea-pigs,[27] and rabbits [14] subsequent to the administration of calcium.

Thus, while the effects of parathyroid hormone and calcium gluconate on cartilage and bone are similar in some respects, they differ in others. As compared with parathyroid hormone, the effects exerted by calcium gluconate were weaker and were demonstrable for shorter periods of time. Moreover, parathyroid hormone caused increased bone formation by means of osteoblastic proliferation. Calcium gluconate, on the other hand, increased the firmness and density of the osseous substance, and thus inhibited its absorption. Finally, the increase of resorptive processes that followed the inhibition of resorption was less prominent in the case of calcium gluconate, whereas it was pronounced in the case of parathyroid hormone.

Notwithstanding the fact that calcium gluconate and parathyroid hormone both accelerated the ageing of the growing epiphyseal cartilage, we did not find an intensification of this effect, if both substances were given simultaneously. This condition may be due to the fact that (1) each substance tends to exert its own peculiar effect on the substratum on which it acts. Consequently, in growing mice, the increased resorption called forth by the parathyroid hormone at later stages is partly counteracted by the inhibiting effect that calcium gluconate exerts on the resorptive processes. Thus, epiphyseo-diaphyseal

union was not only not accelerated, but it was even delayed as compared with the condition found after injections of parathyroid hormone alone. (2) The response of the tissues to hormonal stimuli is partly determined by conditions in the tissues themselves. There is a certain limitation to the tissue response. If the capacity of the cartilage to undergo calcification is exhausted by the administration of parathyroid hormone, an additional supply of calcium does not call forth a further increase in the calcification.

However, the two substances support each other as far as their action on bone is concerned. In this case, a summation effect occurs for a certain length of time; the surplus of calcium available to the proliferating osteoblasts favors the formation of excess bone. Moreover, the inhibition of resorptive processes causes the prolonged persistence of the bone. Thus considerable amounts of excess bone are found at certain experimental stages.

Summary

In mice receiving an adequate amount of dietary calcium, parathyroid hormone promotes the hypertrophy, calcification and disintegration of the growing epiphyseal cartilage without stimulating its proliferation; it intensifies the formation of bone by stimulating osteoblastic proliferation. Subsequently, increased resorption of cartilage and bone causes an accelerated onset of epiphyseo-diaphyseal union. Parathyroid hormone thus promotes the changes that are characteristic of skeletal ageing. In old mice increased calcification of the inactive epiphyseal cartilage is associated with or followed by increased formation of bone.

Calcium gluconate also promotes the ageing of the growing epiphyseal cartilage by increasing disintegration, calcification and ossification. Resorptive processes, however, are temporarily inhibited in both young and adult mice. The effect of calcium gluconate is less marked and of shorter duration than that of parathyroid hormone.

Combined administration of parathyroid hormone and calcium gluconate does not intensify the ageing effect exerted on the growing cartilage by each substance alone. Bone formation, however, is more increased than after administration of each substance alone in both growing and adult mice.

The photomicrographs were made by Mr. Edgar Nebel.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 102

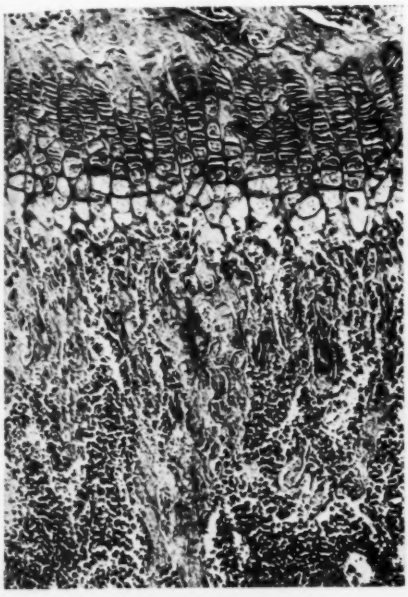
FIG. 1. Epiphyseal disk at the upper tibia of a normal male mouse of strain C57, 7 weeks old. × 130.

FIG. 2. Epiphyseal disk at the upper tibia of a male mouse of strain C57, which, at the age of 6 weeks, had received 4 units of parathyroid hormone and was sacrificed on the next day. There is increased hypertrophy of the epiphyseal cartilage cells. The subchondral layer contains more spicules; the bone marrow between the trabeculae is replaced by an osteogenic tissue. (See Fig. 5.) × 130.

FIG. 3. Epiphyseal disk at the upper tibia of a male mouse of strain C57, which, since the age of 6½ weeks, had received 4 units of parathyroid hormone on 4 successive days, and which was sacrificed 1 day after the last injection. Epiphyseal plate is heavily calcified. As compared with Figure 1, trabeculae are more numerous, thickened and densely calcified. × 130.

FIG. 4. Epiphyseal disk at the upper tibia of a male mouse of strain C57, 10 weeks old, which, since the age of 6 weeks, had received 4 units of parathyroid hormone three times weekly. The greatly narrowed and strongly calcified epiphyseal plate shows perforations leading to communications between the epiphysis and diaphysis. × 130.

1



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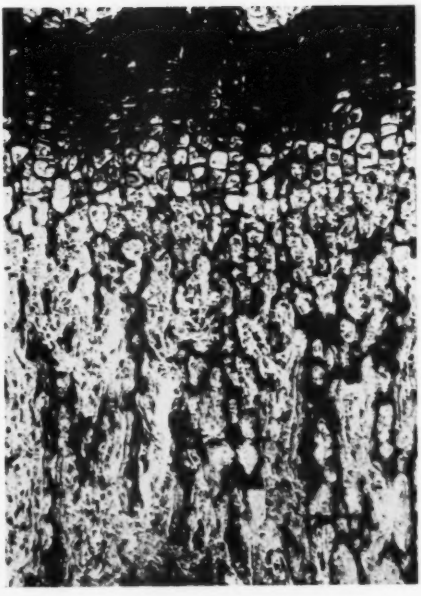

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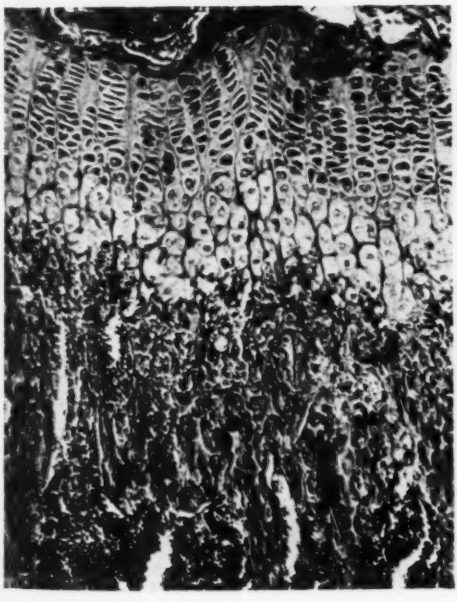

4

Silberberg and Silberberg

Parathyroid Hormone and Calcium Gluconate

PLATE 103

FIG. 5. Area of the subepiphyseal layer of the animal shown in Figure 2. Mitotic proliferation of the peritrabecular cells is indicated by arrows. × 650.

FIG. 6. Area of the shaft of the animal shown in Figure 2. There is increased formation of spindle cells and of osteoblasts at the inner surface of the shaft. × 650.

FIG. 7. Area of the shaft of the animal shown in Figure 3. There is increased formation of osteoblasts at the inner surface. Endosteal connective tissue cells are proliferating. Mitosis is present. No invasion is seen of the proliferating spindle cells into the layer of osteoblasts, nor into the shaft nor the bone marrow. × 650.

FIG. 8. Area of the shaft of the animal shown in Figure 4. The inner surface is shown lined by a thin layer of spindle cells, as seen ordinarily in untreated animals. × 650.

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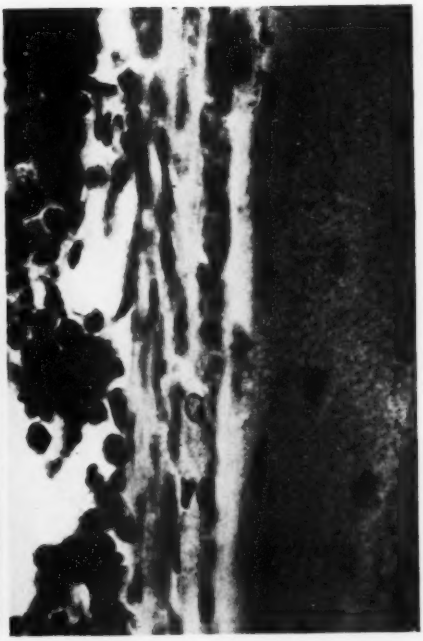

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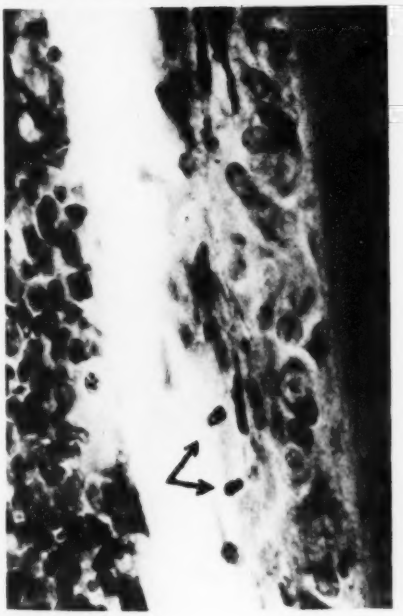

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Silberberg and Silberberg

Parathyroid Hormone and Calcium Gluconate

PLATE 104

FIG. 9. Upper tibia of a normal male mouse of strain CBA, 14 months of age. Remnants of hyalinized inactive epiphyseal cartilage are seen, delimited towards the bone marrow by a thin bony lamella. × 130.

FIG. 10. Upper tibia of a male mouse of strain CBA, 13 months old, which, since the age of 12 months, had received 7 units of parathyroid hormone three times weekly. There is a thickened osseous lamella underneath the heavily calcified cartilaginous remnants of the former growth zone. × 130.

FIG. 11. Epiphyseal disk at the upper tibia of a male mouse of strain C57, 12 weeks old, which, since the age of 8 weeks, had received injections of 0.25 cc. of a 10 per cent solution of calcium gluconate three times weekly. Hyalinized epiphyseal cartilage showing three plugs of degeneration. × 130.

FIG. 12. Upper tibia of a male mouse of strain C57, 14 months old, which, since the age of 12 months, had received alternating injections of 7 units of parathyroid hormone and 0.25 cc. of a 10 per cent solution of calcium gluconate three times weekly. There is heavily calcified cartilage in the former growth zone. Much new bone is laid down in the subepiphyseal layer. × 130.

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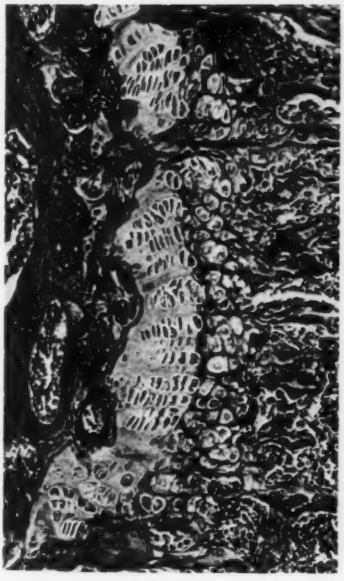

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Parathyroid Hormone and Calcium Gluconate

THE TISSUE CHANGES PRODUCED BY ESTRONE INJECTED INTO FEMALE DOGS WITH BILE FISTULAS *

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The changes produced by large doses of natural and synthetic estrogens in the hematopoietic system, the endocrine system, and the other organs of normal dogs have been described by several investigators.[1-4] The excretion of injected estrogens in the bile of dogs with bile fistulas [5-7] and in the bile obtained from dogs at autopsy [8] has also been investigated. To our knowledge there have been no reports concerning the anatomic findings following the injection of estrogens into dogs with bile fistulas. In a continuation of a recent study [6] in which the biliary excretion of estrogens was determined following the injection of a small amount of estrone, six bitches with bile fistulas were employed to investigate the biliary excretion after large injections of estrone.† The anatomic findings in these animals are reported in this paper.

MATERIALS AND METHODS

The results obtained in three animals which received subcutaneous injections of estrone in sesame oil are compared with those in three control dogs which received sesame oil only. The details of the preparation of the bile fistulas have been reported.[6] Each animal received 50 cc. of bile daily by stomach tube. The controls furnished bile for administration to the experimental animals. The letters, BF, in the protocols designate the experimental animals and the letters, BFC, designate the controls.

Unless otherwise stated in the protocols, macroscopic and microscopic examination was carried out on the following tissues: thymus, thyroid, parathyroids, heart, aorta, lungs, spleen, esophagus, stomach, intestines, liver, gallbladder, pancreas, adrenals, kidneys, bladder, uterine horns, ovaries, vagina, lymph nodes, bone marrow (rib, vertebral, femoral), pituitary, subcutaneous tissue at the sites of injection, and the anterior abdominal wall. Those tissues which were considered to be normal are not described specifically in the summary of the anatomic findings. After fixation in Zenker's solution, the tissues were imbedded in paraffin, cut at 6μ and stained with hematoxylin and eosin. In the case of the spleen, the liver, the lymph nodes and the bone marrow, additional sections were stained with Turnbull's

* Received for publication, December 14, 1942.

† The estrone was obtained through the courtesy of Dr. Erwin Schwenk of the Schering Corporation, Bloomfield, N. J.

blue reaction for the demonstration of iron, and with Giemsa's stain for the study of the cells of the hematopoietic system. Portions of liver and kidneys were also fixed in a 4 per cent solution of formaldehyde, cut at 15μ and stained with scarlet red for the demonstration of neutral fat. The technic of the differential cell count on the rib marrow has been described.[9] The differential count of the blood leukocytes was done on 200 cells of smears stained with the May-Grünwald-Giemsa stain.

Protocols of Experiments

Dog BF-3. Weight, 23 lbs. Beginning on the eighth day after operation this dog received daily, for 15 days, 5 mg. of estrone in 1 cc. of sesame oil, a total of 75 mg. Death occurred 2 days after the last injection. Just before death she developed marked lassitude, and vaginal bleeding occurred. There were numerous petechiae in and around the mouth. Her final weight was 18 lbs.

Macroscopic examination revealed the following: a pale, dilated, flabby heart with petechiae on the surface of the right auricle; multiple petechiae and ecchymoses throughout the lungs; a brown-gray-green mottled and friable liver; a thickened, opaque gallbladder; a large right uterine horn and a left uterine horn distended with blood; large, congested mediastinal and mesenteric lymph nodes; pale, tan-gray rib marrow; and anterior abdominal wall tissues which were dark red, swollen and friable.

Microscopic examination disclosed the following: infiltration of the stroma of the right auricle and of the sinusoids of the spleen, liver (Fig. 1), adrenals and lymph nodes by large numbers of cells of the neutrophile granulocyte line varying from promyelocyte to segmented forms; small foci of bronchopneumonia; large amounts of granular, brown, iron-containing pigment in the macrophages and reticulo-endothelial cells of the spleen, liver, lymph nodes and bone marrow; amorphous, brown, iron-free pigment in most of the canaliculi of the liver (Fig. 7); dilatation of the basal glands of the endometrium with increased height of the lining epithelium in which were inclosed nuclear débris and polymorphonuclear neutrophils, focal edema and infiltration of the stroma around the basal glands by polymorphonuclear neutrophils, dilatation of the tubules which were lined with flattened epithelium and in which cellular débris was contained, and infiltration of the crypt layer by lymphocytes, plasma cells, and monocytes; tubes lined by high-columnar ciliated epithelium; ovaries which were about 2 months metestrum—many of the lutein cells of the corpora lutea contained nuclei with between one and five acidophilic inclusion bodies

(Fig. 8), which were distinct from the smaller basophilic nucleoli; a greatly increased myeloid/erythroid ratio, a marrow cell/fat cell ratio of 100/1, and an absence of megakaryocytes in the rib marrow (Fig. 5); and an acute necrotizing, hemorrhagic cellulitis and myositis of the anterior abdominal wall. The determination of the cellular elements of the peripheral blood and a differential count of the cells of the bone marrow were not done for this animal.

Dog BF-4. Weight, 25 lbs. Beginning on the sixth day after operation this dog received daily, for 15 days, 5 mg. of estrone in 1.5 cc. of sesame oil, a total of 75 mg. She was sacrificed on the third day following the last injection. Her final weight was 24 lbs.

Macroscopic examination revealed the following: a pale, flabby heart with petechiae in the epicardium of the right auricle; ecchymoses over the upper lobe of the right lung; brown yellow-green, moderately firm liver; thickened, gray, red mottled gallbladder; gray-red rib and vertebral marrow and yellow, red-stained femoral marrow; and swollen and dark red tissue of the anterior abdominal wall.

Microscopic examination disclosed the following: petechial hemorrhages and small foci of segmented neutrophils in the stroma of the right auricle; petechial hemorrhages and small foci of organizing pneumonia in the lungs; moderate hyperplasia of the lymphatic tissue, and sinusoidal macrophages inclosing granular, brown, iron-containing pigment in the spleen and the lymph nodes; granular, brown, iron-containing pigment in the Kupffer cells of the liver, amorphous, brown, iron-free pigment in many of the bile canaliculi, and fatty metamorphosis of the periportal liver cells; chronic cholecystitis; tall-columnar epithelium lining the basal glands, tubules, crypts and surface, and an edematous crypt layer in the endometrium; tall-columnar ciliated epithelium lining the tubes; ovaries about 2 months anestrum; a lining of stratified squamous epithelium six to seven layers thick and edema of the lamina propria in the vagina; acute, diffuse cellulitis and fibrosis of the anterior abdominal wall; and large empty spaces surrounded by flattened, foamy macrophages, segmented neutrophils, fibrinoid necrotic material and fibrous connective tissue at the site of injection.

Dog BF-6. Weight, 25.7 lbs. Beginning on the 25th day after operation this dog received 5 mg. of estrone in 1.5 cc. of sesame oil daily for 7 days and 6.3 mg. on the 8th day, 41.3 mg. in all. She was sacrificed on the 3rd day after the last injection. The final weight was not recorded.

Macroscopic examination showed the following: a brown, firm liver; a thickened, opaque gallbladder; gray-brown rib marrow, dark red

vertebral marrow and yellow-brown femoral marrow; a gray, indurated anterior abdominal wall; and thickened, boggy, yellow-gray injection sites.

Microscopic examination revealed the following: granular, brown, iron-containing pigment in the macrophages and reticulo-endothelial cells of the spleen, liver, lymph nodes and bone marrow; amorphous, brown, iron-free pigment in the bile canaliculi of the liver; chronic cholecystitis; basal glands lined by tall-columnar epithelium, edema of the stroma, and numerous pigmented macrophages in the endometrium; ovaries in late metestrum; a lining of stratified squamous epithelium and edema of the lamina propria in the vagina; proliferated patches of stratified squamous epithelium from the basal layer (with crowding away of the overlying columnar cells), edema and pigmented macrophages in the lamina propria of the endocervix; and reactions at the injection sites in which the early response showed segmented neutrophils, fibrin, granulation tissue, fresh hemorrhage, connective tissue proliferation and pigmented macrophages, and in which the late response consisted of foreign body giant cells, fibrin, pigmented macrophages, and fibrous connective tissue.

Dog BFC-1. Weight, 15 lbs. Beginning 1 day after operation this dog received 1.0 cc. of sesame oil daily for 21 days. During this time part of her bile was used for feeding a dog with a bile fistula which received estrone, but which was not necropsied. Beginning on the 25th day after operation she was given 1.0 cc. of sesame oil daily for 29 days. She was sacrificed 3 days after the last injection when her weight was 9 lbs.

Macroscopic examination revealed the following: a thickened, gray gallbladder; gray-red rib and vertebral marrow and yellow, red-streaked femoral marrow; and moderately thickened, gray anterior abdominal wall.

Microscopic examination disclosed the following: a few pigmented macrophages in the thymus; slight fatty metamorphosis of the liver cells in the central part of the lobules (Fig. 2); chronic cholecystitis; the uterine horns, ovaries, and vagina in early anestrum; and normoblastic hyperplasia in the rib marrow (Fig. 6).

Dog BFC-3. Beginning 2 days after operation this dog received 1.5 cc. of sesame oil daily for 10 days. She was sacrificed on the tenth day.

Macroscopic examination revealed the following: a pale, tan-red, firm liver; a thickened, opaque gallbladder; dark red rib and vertebral marrow and tan femoral marrow; and a gray, thickened anterior abdominal wall.

Microscopic examination showed the following: varying amounts of granular, brown, iron-containing pigment in the macrophages and reticulo-endothelial cells of the spleen, liver, lymph nodes and bone marrow; amorphous, brown, iron-free pigment plugging some of the bile canaliculi; moderate vacuolation of the liver cells in the central part of the lobules; focal acute cholecystitis; and immature uterine horns, ovaries and vagina.

Dog BFC-4. Beginning 2 days after the operation this dog received 1.5 cc. of sesame oil daily for 9 days. She was sacrificed on the ninth day.

Macroscopic examination disclosed the following: a pale, tan, firm liver; a thickened, opaque gallbladder adherent to the duodenum; dark red rib and vertebral marrow and light tan-red femoral marrow; and a thickened, gray anterior abdominal wall.

Microscopic examination disclosed the following: small foci of bronchopneumonia; chronic cholecystitis; immature uterine horns, ovaries and vagina; acute lymphadenitis of the mediastinal lymph nodes, and scattered pigmented macrophages in the sinusoids of two mesenteric lymph nodes; and many clear spaces surrounded by one or more rows of monocytes at the site of injection.

Comment

In Table I is given a comparison of the results of the staining reactions for neutral fat in the liver and kidney, and for pigment in the liver, spleen, lymph nodes and bone marrow. The tissues of the animals which received estrone in oil revealed certain changes which are possibly due to the injections of estrone. The neutral fat content of the liver cells does not appear to differ greatly in the experimental or con-

Table I

Neutral Fat in Liver and Kidney and Pigment in Spleen, Liver, Lymph Nodes and Bone Marrow

	Animals injected with estrone in sesame oil			Animals injected with sesame oil alone		
	BF-3	BF-4	BF-6	BFC-1	BFC-3	BFC-4
Neutral fat in liver cells	o	II	o	II	III	o
Neutral fat in cells of cortical portion of renal collecting tubules (Figs. 3 and 4)	V	V	III	I	II	o
Iron-containing pigment in Kupffer cells of liver	V	IV	II	I	II	o
Iron-containing pigment in bone marrow	V	IV	V	I	II	I
Iron-containing pigment in spleen	V	III	III	I	III	I
Iron-free pigment in canaliculi of liver	V	V	II	o	II	o
Iron-containing pigment in lymph nodes	V	IV	V	II	IV	III

Grade I indicates presence; grade V, abundance; and grades II, III, and IV, intermediate amounts of neutral fat and pigment.

trol animals. However, there is an increased amount of neutral fat in the cells of the cortical portions of the renal collecting tubules in those animals which received estrone in oil as compared with those which received oil only. The deposit of iron-containing pigment is definitely greater in the Kupffer cells of the liver, in the bone marrow and in the spleen of those animals which received estrone. The greater quantity of iron-free pigment in the bile canaliculi of the livers of the animals injected with estrone is readily apparent. The amount of iron-containing pigment in the lymph nodes does not differ significantly in the two groups.

Table II contains the data for the last blood picture of all animals except dog BF-3. A striking leukocytosis and shift to the left in the neutrophile granulocyte line is seen only in dog BF-4, probably because this animal received almost twice as much estrone as dog BF-6.

Table III shows the results of the differential count of the cells of the

TABLE II

Data of the Last Examination of the Blood

	Experimental dogs		Control dogs		
	BF-4	BF-6	BFC-1	BFC-3	BFC-4
Hemoglobin (Newcomer), gm.	15.6	12.0	9.2	14.2	11.6
Erythrocytes—millions per cmm.	6.53	4.80	4.64	6.0	5.55
Leukocytes per cmm.	44100	28050	10950	11550	9650
Metamyelocyte neutrophils, %	8.5	0.5	0.0	1.0	3.0
Stab neutrophils, %	40.0	14.5	4.0	14.5	19.0
Segmented neutrophils, %	36.5	70.5	73.0	80.0	56.0
Lymphocytes, %	12.0	12.0	15.0	2.5	20.5
Eosinophils, %	2.0	2.5	7.0	0.5	1.0
Monocytes, %	1.0	0.0	1.0	1.5	0.5

TABLE III

Differential Counts of the Rib Marrow; Cellularity of the Rib, Femoral, and Vertebral Marrows; and Number of Megakaryocytes in the Bone Marrow

	Experimental dogs		Control dogs		
	BF-4	BF-6	BFC-1	BFC-3	BFC-4
Eosinophils, %	1.6	3.2	4.8	2.0	5.4
Promyelocyte neutrophils, %	8.6	4.0	0.4	3.0	1.0
Myelocyte neutrophils, %	24.0	22.2	3.6	12.8	10.2
Metamyelocyte neutrophils, %	34.6	28.2	7.4	16.4	20.2
Stab neutrophils, %	19.6	24.2	14.4	26.8	23.4
Segmented neutrophils, %	0.0	0.8	2.6	5.8	2.8
Pro-erythroblasts, %	0.0	0.2	1.6	0.6	0.2
Erythroblasts, %	1.0	1.6	3.6	3.4	2.0
Normoblasts, %	6.2	11.2	57.6	21.6	29.2
Stem cells, %	0.6	0.6	0.6	0.4	0.0
Lymphocytes, %	1.8	1.2	2.0	3.8	3.4
Unidentified cells, %	2.0	2.6	1.4	3.4	2.2
Myeloid/erythroid ratio	12.3	6.4	0.5	2.6	2.0
Marrow cell/fat cell ratio					
rib	100/1	75/25	90/10	65/35	75/25
vertebra	100/1	75/25	90/10	65/35	65/35
femur	.....	30/70	35/65	10/90	25/75
Megakaryocytes	Absent	Absent	Many	Many	Many

rib marrow, the cellularity of the rib, vertebral and femoral marrows, and the number of megakaryocytes in the bone marrow for all animals except dog BF-3. The rib marrows of dogs BF-4 and BF-6 reveal a marked shift to the left in the neutrophile granulocyte line, a great increase in the myeloid/erythroid ratio, hyperplasia, and an absence of megakaryocytes. These observations confirm those of other workers.[1-3]

The hyperplasia and normoblastic activity in the marrow of dog BFC-1 may be related to the length of time she carried the bile fistula even though she was fed presumably adequate amounts of bile by stomach tube. The presence of anemia in dogs with bile fistulas has been recently demonstrated.[10]

The inflammatory changes in the gallbladder, the fibrosis of the anterior abdominal wall and the fibrosis around the bile drainage tube with the formation of a sinus tract were necessary results of the operative procedure.

In the animals injected with estrone, the high-columnar ciliated epithelium lining the tubes, the evidences of glandular hyperplasia of the endometrium, the edema of the endometrium and of the lamina propria of the vagina, the proliferation of a stratified squamous epithelium in the vagina, the pigmented macrophages in the endometrium, and possibly the intranuclear inclusion bodies in the lutein cells of dog BF-3 may be considered to be results of the action of estrone. Changes of this type were not found to be a part of the normal histologic variations in the ovaries, uterine horns, or vagina.[11]

Under the conditions of the experiments, the injections had no effect on the pituitary, thyroid, parathyroids, adrenals, or pancreatic islets as judged by the absence of definite histologic alterations in these glands.

The mild inflammatory changes seen in the lungs appear to have no significance when the findings in the two groups are compared.

Summary

The anatomic findings following the injection of estrone in sesame oil into three bitches with bile fistulas were compared with those in three similar animals injected with sesame oil alone. In contrast to the dogs injected with sesame oil alone, those injected with estrone in oil showed the following tissue changes:

1. An increased amount of fat in the cells of the cortical portions of the renal collecting tubules;

2. A greater amount of iron-containing pigment in the Kupffer cells of the liver, in the bone marrow and in the spleen;

3. A larger quantity of iron-free pigment in the bile canaliculi of the liver;

4. Stimulation of granulocytopoiesis and suppression of megakaryocytosis confirming the observations of other investigators;

5. Evidence of estrogenic stimulation of the endometrium, endocervix and vagina;

6. Intranuclear inclusion bodies in the lutein cells of the ovaries of one animal.

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[*Illustrations follow*]

DESCRIPTION OF PLATE

PLATE 105

FIG. 1. Liver of dog BF-3. Many young forms of the neutrophile granulocyte line are present in the sinusoids. Hematoxylin and eosin stain. × 120.

FIG. 2. Liver of dog BFC-1. Sinusoids are practically devoid of leukocytes as compared with Figure 1. There is a fine vacuolation of cytoplasm of many liver cells, a characteristic of abundant glycogen. Coarse fat vacuoles are seen in several liver cells. Hematoxylin and eosin stain. × 120.

FIG. 3. Kidney of dog BF-3. Black granules represent neutral fat in the cells of the cortical portions of the collecting tubules. Scarlet red stain. × 120.

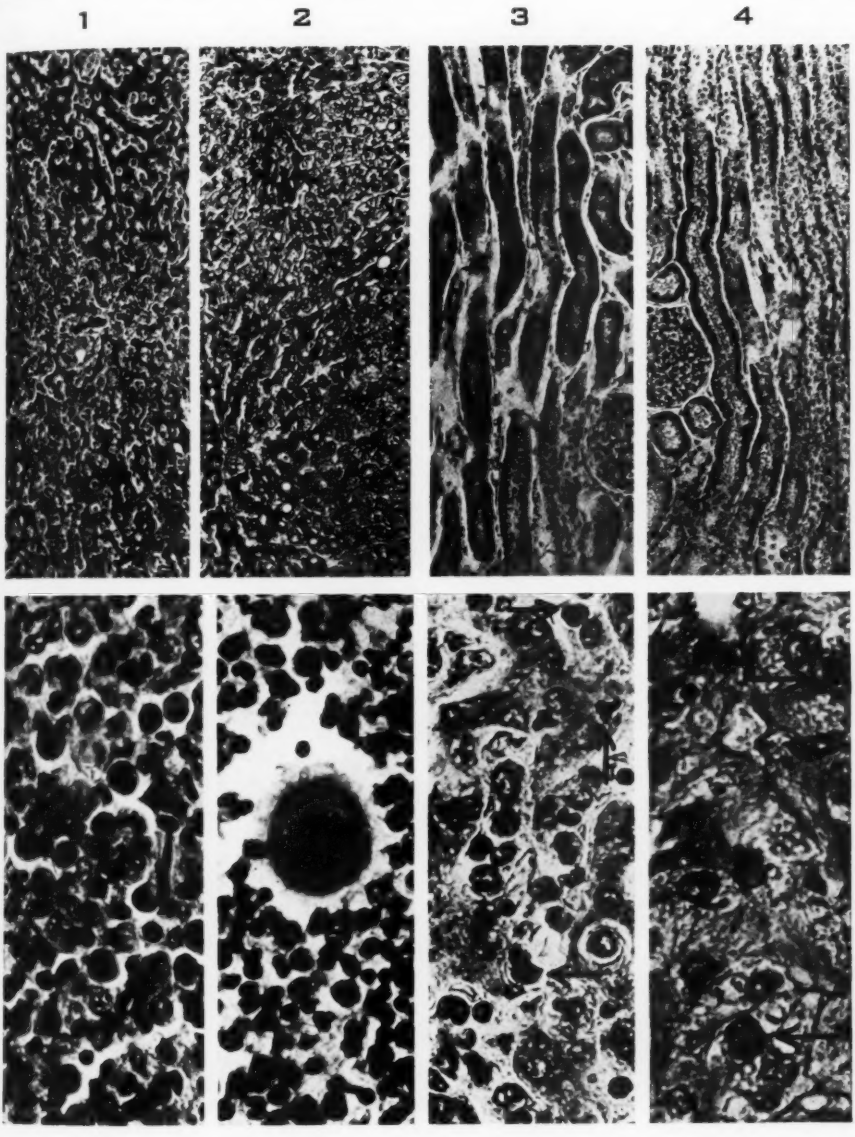
FIG. 4. Kidney of dog BFC-1. Black granules represent neutral fat in the cells of the cortical portions of the collecting tubules. Scarlet red stain. × 120.

FIG. 5. Rib marrow of dog BF-3. Representative area showing marked hyperplasia, absence of megakaryocytes and preponderance of young forms of the neutrophile granulocyte line. Hematoxylin and eosin stain. × 600.

FIG. 6. Rib marrow of dog BFC-1. Representative area showing hyperplasia, many normoblasts, a well preserved megakaryocyte and more mature forms of the neutrophile granulocyte line. Hematoxylin and eosin stain. × 600.

FIG. 7. Liver of dog BF-3. Many young forms of the neutrophile granulocyte line in the sinusoids. Arrows in the upper half of the field point to bile canaliculi plugged with pigment. The arrow in the lower half points to a Kupffer cell loaded with pigment. Hematoxylin and eosin stain. × 600.

FIG. 8. Corpus luteum, ovary of dog BF-3. The arrow in the upper half of the field points to a lutein cell nucleus with two inclusion bodies. The arrow in the lower half points to a lutein cell nucleus, with inclusion body in the lower portion and nucleolus in the upper portion. Hematoxylin and eosin stain. × 600.



Mulligan, Longwell and Morrell Estrone in Dogs with Bile Fistulas

THE NATURE OF THE HYALINE MATERIAL IN THE PANCREATIC ISLANDS IN DIABETES MELLITUS *

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The histopathology of diabetes mellitus, although extensively investigated, must still be considered an unconquered territory since, of the many changes described, none has as yet been proved conclusively to be the responsible manifestation of diabetes. Most of the reports on this subject have failed to differentiate between adult and juvenile diabetes which, of course, has added to the confusion. Keilty [1] and Weichselbaum and Stangl [2] considered grossly recognizable pancreatic atrophy a significant factor in diabetes mellitus, while Cecil [3] found a reduction in the size of the pancreas in only 25 per cent of his cases. Warren,[4] in a study of 484 diabetic patients, reported the following pancreatic changes: hyalinization, 200 cases; fibrosis, 129; normal pancreas, 127; hydropic changes, 22; lymphocytic infiltration, 9; calculi, 3; amyloid, 2. Martius [5] reported the following island changes in diabetes mellitus in order of frequency: sclerosis, hyalinization, hydropic degeneration, simple atrophy, hemorrhage. Interstitial pancreatitis was found by Gibb and Logan [6] in 123 of 147 cases of diabetes mellitus. Herzog [7] interpreted multiple fibrous nodules in the pancreas of a diabetic person as remnants of islands. Wilder [8] called attention to the more frequent occurrence of lipoids in the islands of diabetic persons than of those without diabetes. Boldyreff [9] reported a case of occlusion of the pancreatic duct resulting in death in diabetic coma years later; the pancreas in this case showed extreme atrophy with disappearance of the island tissue. In contrast to this report, Umber,[10] in a very similar case, found marked regeneration of the islands without existence of diabetes mellitus. A segregation of cases of juvenile diabetes from those of diabetes of the higher age groups was made by Glen,[11] who found island changes to be rare in the former and frequent in the latter group. Labbé and Pétresco [12] found sclerosis of the islands more frequent in the middle-aged group while hydropic and pyknotic islands were more predominant in the younger patients.

Purpose of Present Investigation

The purpose of this investigation is to demonstrate that the term "hyalinization" in reference to the islands of Langerhans is misleading since the hyaline material frequently found in the pancreatic islands

* Received for publication, December 31, 1942.

shows staining properties identical with those of amyloid. If special stains are employed, it can be demonstrated that these changes are extremely frequent and appear where routine hematoxylin and eosin stains have failed to reveal any pathology.

Only a few reports in the literature mention amyloid in reference to the pancreatic islands. Opie,[13] in 1900, reported a case of hyaline degeneration of the pancreatic islands in which he mentioned specifically that the reactions for amyloid were negative for this hyaline material. In 1905, Reitmann[14] reported two fatal cases of pulmonary tuberculosis in a middle-aged male and a young female in whom amyloid was found in the islands of Langerhans but nowhere else; the author's description suggests that the process in these cases was histologically indistinguishable from hyalinization of the islands. In 1937, Warren[4] pointed out that hyalinization of the islands is caused by the deposition of a substance which is "related to or possibly identical with amyloid." In his review of pancreases of 484 diabetic persons he found hyaline islands in 200, with 97 per cent in persons over 40 years of age. Of 51 of these cases, tissue from 14 gave positive staining reactions for amyloid. Although Warren suggested the amyloid theory of insular hyalin, he expressed doubts as to its value because of negative amyloid stains in the majority of his cases.

In contrast to Warren's[4] observations, none of the cases presented in this investigation failed to show positive amyloid stains in the hyaline material deposited in the pancreatic islands. Van Gieson's stain gave the same shade of yellow which it produced on known amyloid tissue. The same van Gieson's mixture applied to ovarian corpora fibrosa yielded the usual pink stain of hyaline connective tissue. The regularity with which the hyaline material of the pancreatic islands gave a positive stain for amyloid seems to prove that this substance is at least related to, and in all probability, identical with, amyloid. Lacking conclusive microchemical means of identification, it seems justified to consider this hyaline material to be amyloid until it can be disproved as such.

Material and Methods

The present material * was derived from 157 autopsied cases: 105 middle-aged or old diabetic patients; 26 juvenile diabetic patients; and 26 nondiabetic patients with island changes found upon routine microscopic examination. The material consisted of formaldehyde-fixed paraffin blocks, usually two or three but in a few cases only one, from each pancreas. From every block sections were stained in hematoxylin

* Obtained from the Department of Pathology, University of Michigan, Ann Arbor, Michigan.

and eosin and with special stains for amyloid. It was found that the most practical method for the demonstration of amyloid was staining with gentian violet followed by differentiation in diluted acetic acid. After proper differentiation the slides were rinsed in tap water and examined immediately while they were still wet, without the use of coverslips. By this method the hyaline material stood out as a brilliant red substance, facilitating detection of even the smallest number of hyaline islands. Rechecks later on, however, required complete re-

TABLE I

Number of Patients with and without Amyloid in the Islands of Langerhans

	Amyloid-positive	Amyloid-negative	Total
Juvenile diabetic patients	1	25	26
Middle-aged or old diabetic patients	67	38	105
Nondiabetic patients with recorded islet pathology	26	..	26
Nondiabetic patients over 50 years of age selected from consecutive autopsy cases	5	45	50

staining. The microscopic studies were made with artificial light, as the natural light decreased the brilliancy of the red color, giving the hyaline material a slightly bluish shade and making its differentiation from the surrounding blue tissue more difficult.

It was found that the occurrence of amyloid varied from one amyloid island in a single level of the pancreas to complete amyloidosis of almost all islet structures. Accordingly, it was attempted to classify these changes arbitrarily by the designation of 1, 2, and 3 plus. Obviously, this quantitative classification was of but little accuracy as it was based on a more or less subjective impression and on the examination of only one, two, or, at the most, three levels of the pancreas; however, for practical purposes it seemed to suffice. Quantitative amyloid changes within individual islets were also noted but not specifically evaluated.

RESULTS OF CASE STUDY

Pancreases of 131 diabetic persons were studied, 26 being cases of juvenile diabetes. Of the 105 middle-aged or older diabetic patients, the pancreases of 67, or 64 per cent, showed amyloid in the islands of Langerhans to varying degrees. Of the 26 juvenile diabetic cases, only 1 pancreas showed amyloid. In addition, the pancreases of 26 nondiabetic patients were examined for which, in the routine microscopic examination of the organs, hyaline changes of the islands were recorded; all of these 26 gave positive amyloid stains of the hyaline material. In-

asmuch as these amyloid-positive nondiabetic cases did not give any information as to the total incidence of this process, the pancreases of 50 consecutive autopsy cases of nondiabetic patients over 50 years of age were examined. Five of these cases (10 per cent) revealed the same amyloid changes, although the involvement was usually slight.

It did not seem justified to separate middle-aged and old diabetic patients, as there are no grounds for such separation from a pathologic point of view. The series of juvenile diabetes was omitted from further investigation as these cases were believed to represent a different disease entity, clinically as well as pathologically. It is true that insular amyloid does occur in cases of juvenile diabetes but these cases are, apparently, rare. In one such instance found in this material, as well as in two similar cases reported by Reitmann,[14] the patients died from advanced pulmonary tuberculosis, which might account for the presence of amyloid in the pancreatic islands.

Clinical Aspects of Investigated Cases

An attempt was made to investigate the clinical relationship between cases with and those without amyloid by a study of the case histories. In this attempt a number of difficulties were encountered because in many instances I had to deal with terminal conditions. Unless the patient had been under observation for some time, the report of a low blood pressure shortly prior to death was of no value; in some cases a left-sided cardiac hypertrophy recorded in the autopsy protocol had to be considered as an indication of a pre-existing hypertension. More difficult was the important evaluation of the body weight, as an apparent weight loss was not always properly recorded. Duration of the disease in most cases had to be estimated from the appearance of suggestive symptoms, such as polyuria or polydipsia, as recorded in the patient's history.

Table II is self-explanatory. While in general the findings offer no conclusive clues in regard to the relationship between amyloid in the islands of Langerhans and diabetes mellitus, there are a few points of interest: (1) Obesity is more common in the diabetic patient with than without amyloid. (2) Amyloid changes in nondiabetic patients are not very common in females. (3) Gangrene of the lower extremities is as common in diabetic patients with amyloid changes as in those showing none. (4) Racial disposition is of no significance in this investigation. (5) Thyroid disease is found in some diabetic persons without amyloid. (6) Hypertension is surprisingly common in connection with amyloid pancreatic islands.

It was noted that the average age of the obese amyloid-positive pa-

tients regardless of absence or presence of diabetes mellitus was distinctly lower than that of the nonobese amyloid-negative diabetic patients. This point may throw an interesting light on the problem of "glycosuria of the obese middle-aged individual"; there is apparently a greater tendency for patients in this group to show amyloid in the pancreatic islands than for those in the group of nonobese older persons.

The duration of the diabetic condition has, apparently, no influence on the presence or absence of pancreatic amyloid. Dry and Tessmer's [15]

TABLE II
Clinical Aspects of Investigated Cases

	Diabetic patients with amyloid in islands	Diabetic patients without amyloid in islands	Nondiabetic patients with amyloid in islands
Number of cases	67	38	26
Males	27	19	22
Females	40	19	4
Percentage of obesity ("obese" or weight of 180 lbs. or more)	54%	36%	45.4%
Average age of "obese" patients	59.3 yrs.	58.3 yrs.	59.2 yrs.
Average age of "nonobese" patients	64 yrs.	59.2 yrs.	65 yrs.
Percentage of patients with systolic blood pressure of 150 or more	40%	28%	50%
Average duration of diabetes	6.2 yrs.	5.7 yrs.	
Previous insulin treatment	14	14	
Diabetes in family	5	5	2
Racial factors	1	3	0
Gangrene of feet or legs	14	8	2
Infections without gangrene	15	6	3
Evidence of thyroid disease	1	3	1

report on an increase in hyaline changes in the post-insulin era over the pre-insulin era cannot be confirmed from the standpoint of the present investigation. Familial history of diabetes does not seem to favor nor exclude the development of amyloid.

Amyloid in the Pancreas in Generalized Amyloidosis

In 11 cases of generalized amyloidosis in nondiabetic persons the pancreases were examined for amyloid. Nine of these cases showed no amyloid in the islands while 2 showed slight involvement (1 plus); all but 2, however, showed amyloid in the arterioles. A second series of 11 cases, all 3 plus positive for amyloid in the islands, was selected from the original material and examined for amyloid in liver, kidneys, adrenals and spleen. Except for amyloid changes in the pancreatic arterioles, no amyloid was found outside of the islands in any of these cases. Amyloid deposits in the small arteries of the pancreas were commonly found in all cases in this investigation. They were observed in many amyloid-positive diabetic and nondiabetic patients and in a

considerable number of diabetic persons who were negative for amyloid in the islets.

Quantitative Amyloid Changes

Results of an attempt to correlate clinical data with the various degrees of islet amyloidosis are shown in Table III. The data obtained did not reveal differences that could be considered significant, but it must be emphasized again that this classification into three groups is based merely on arbitrary standards and on the findings in a limited

TABLE III

Clinical Aspects of Investigated Cases Arranged According to Degrees of Amyloidosis of the Islands of Langerhans

	Diabetic patients				Nondiabetic patients		
	No amyloid	One plus	Two plus	Three plus	One plus	Two plus	Three plus
Average age in years	58.3	60	60	57	60	62	66.5
Males	19	5	11	11	3	8	10
Females	19	16	14	11	1	1	2
Weight of 180 lbs. and over	23	10	13	6	1	5	4
Weight of less than 180 lbs.	13	9	9	13	4	8	10
Blood pressure of 150 and more systolic	11	10	13	7	2	3	7
Blood pressure of less than 150 systolic	21	7	10	12	2	4	4
Insulin treatment	14	4	2	8			
No insulin treatment	14	12	16	13			
Average duration of diabetes in years	5.5	6.2	5.9	8.3			
Gangrene	8	3	6	5			2
No gangrene	30	16	19	17			10

number of levels of the pancreas. It does not rule out the likelihood that systematic studies, if they were possible, might shift the data of Table III. In the material available for investigation a quantitative classification seems without value.

COMMENT

The principal findings in this investigation are as follows: (1) The term "hyaline fibrosis" of the islands of Langerhans is a morphologic misinterpretation. The hyaline substance found frequently in the pancreatic islands is not connective tissue hyalin but, because of its staining reactions, is believed to be amyloid. (2) Sixty-four per cent of middle-aged or older diabetic patients and 10 per cent of nondiabetic patients of 50 years or over show amyloid of varying degree in the islands of Langerhans. (3) Amyloid is found isolated in the islands without amyloid involvement of other organs, while in cases of generalized amyloidosis the islands are usually not involved at all. (4) Hyper-

tension is commonly found in amyloid-positive diabetic and nondiabetic patients.

Inasmuch as amyloid is more than six times as common in the pancreases of diabetic persons as of nondiabetic persons, a relationship between insular amyloid and diabetes mellitus seems probable. In determining the nature of this relationship it should not be forgotten that: (1) approximately one-third of all cases of diabetes mellitus do not show insular amyloid; (2) in some amyloid-positive cases the number of involved islands is extremely small; and (3) a certain number of persons show insular amyloid without being diabetic. It seems impossible to reconcile these facts with the theory that insular amyloid causes diabetes mellitus. It seems more reasonable to consider amyloid degeneration the result rather than the cause of diabetes and to assume that the pancreatic islands may or may not undergo such degeneration subsequent to pre-existing nondemonstrable changes. Whether the nondiabetic group with insular amyloid should be regarded as being potentially diabetic, as suggested by Warren,[4] is difficult to determine as long as we have to deal with post-mortem material. Some resemblances to the amyloid-positive diabetic series, such as the high incidence of hypertension, examples of familial history of diabetes and the occurrence of gangrene of the lower extremities, might be considered as supporting such a statement.

The large number of hypertensive cases in the amyloid-positive, nondiabetic series seems quite surprising. Offhand no apparent relationship can be elicited between these two findings from a physiologic point of view. Looking at the problem from a purely histologic angle, however, it may prove of interest to consider the resemblance between renal glomeruli and islands of Langerhans and the relationship of the former to hypertension. It is only one step further to suggest that a pathologic condition of the highly vascular islands of Langerhans may interfere with the circulation very much as do diseased renal glomeruli. The affinity of amyloid for small blood vessels supports this conception in so far as the amyloid seems to be laid down about the endothelial structures first.

If I am correct in my assumption of a relationship between diabetes mellitus and insular amyloid, the question comes up: what causes these nondemonstrable changes in diabetes mellitus which may or may not be followed by amyloid degeneration of the islands of Langerhans? Considering juvenile diabetes a disease entity of a separate nature, diabetes mellitus is a disease of the middle-aged or older person, which points to a possible arteriosclerotic etiology. Weichselbaum and Stangl[2] found arteriosclerosis of the pancreatic vessels in diabetic persons over

50 years of age. Allen [16] stated that he does not believe that a decrease of arterial blood supply can cause diabetes mellitus. Warren [4] emphasized that in most cases of diabetes mellitus the pancreatic vessels are of sufficient caliber to insure adequate blood supply.

It seems that the vascular etiology of diabetes mellitus cannot be discussed merely on the basis of absence or presence of sclerosis of pancreatic arteries and arterioles but that the blood supply of the pancreas as a whole should be considered. With the bulk of the islet tissue lying in the tail of the pancreas, it should be kept in mind that the head and the tail of the pancreas draw their blood supplies from two different sources: while the head is largely supplied by the superior and inferior pancreaticoduodenal arteries, the tail receives most of its blood through the pancreatic branches of the splenic artery. Thus, the vascular island tissue draws its blood supply mostly from the splenic artery. It is quite conceivable that arteriosclerosis of the splenic artery might greatly interfere with adequate blood supply to the tail of the pancreas by blocking the rather small openings of the pancreatic branches. Thus, this interference may take place outside of the pancreas without manifestations of arteriosclerosis in the more distal portions of the pancreatic blood supply. A relationship between insular amyloid and the splenic circulation is suggested by Warren's [4] observation of amyloid in the splenic arterioles in those of his cases which showed positive amyloid reactions in the hyalinized islands.

Summary

1. The change in the islands of Langerhans known as hyaline fibrosis has been misinterpreted histologically and, in all probability, is a deposition of amyloid.

2. In 67 of 105 middle-aged and older diabetic persons (64 per cent), amyloid deposition of varying degrees could be demonstrated in the islands of Langerhans.

3. Similar changes were found in 5 out of 50 consecutive autopsies upon nondiabetic patients over 50 years of age.

4. Amyloidosis of the islands of Langerhans is an isolated feature and, as a rule, is not found in generalized amyloidosis.

5. Amyloidosis of the islands of Langerhans cannot be considered a pathognomonic evidence of diabetes mellitus nor its cause. It is suggested that nondemonstrable changes in diabetes mellitus and, possibly, in pre-diabetic states render the islands susceptible to amyloid deposition.

6. In a high percentage of cases amyloidosis of the islands of Langerhans in both diabetic and nondiabetic persons is associated with

hypertension. The morphologic resemblance between islands of Langerhans and renal glomeruli suggests interference with the circulation in the highly vascular islands as a possible cause of increased blood pressure, in a manner comparable to that resulting in renal hypertension.

7. The percentage of obese patients is slightly higher and their average age lower in the amyloid-positive than in the nonamyloid group; in other words, there is a greater tendency in "glycosuria of the middle-aged obese individual" towards insular amyloidosis than in the older, nonobese patient.

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After this paper was set in type, the article by J. B. Arey (Nature of the hyaline changes in islands of Langerhans in diabetes mellitus. *Arch. Path.*, 1943, **36**, 32–38) appeared. Both results and conclusions are in substantial agreement with those reported here.

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EFFECT OF VITAMIN E THERAPY ON THE CENTRAL NERVOUS SYSTEM IN AMYOTROPHIC LATERAL SCLEROSIS *

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Until recently it was universally recognized that amyotrophic lateral sclerosis is not amenable to any form of treatment. Wechsler,[1] on the basis of experimental studies of animals that were deprived of vitamin E and which developed paralysis and atrophies (Ringsted,[2] Lipshutz,[3] Burr, Brown and Moseley,[4] Einarson and Ringsted[5]), believed that patients suffering from amyotrophic lateral sclerosis might respond to a synthetic preparation containing alpha-tocopherol. Wechsler,[1] Rosenberger[6] and Bicknell[7] found that alpha-tocopherol and natural vitamin E act favorably in some cases of amyotrophic lateral sclerosis and bring about varying degrees of improvement, perhaps in inverse ratio to the age and duration of the disease. Doyle and Merritt,[8] Denker and Scheinman[9] and Ferrebee, Klingman and Frantz,[10] however, using the same form of treatment, were unable to produce improvement or to arrest the course of the illness in patients with amyotrophic lateral sclerosis.

A number of cases of amyotrophic lateral sclerosis were treated at the Montefiore Hospital with vitamin E and alpha-tocopherol. Ten of these cases came to necropsy and, except for one, none had responded clinically to this form of treatment. The age, duration of the illness and the fact that they may not have been treated adequately may be used as arguments against the lack of improvement. In this presentation, emphasis will be placed on the possible influence of this form of treatment on the affected structures of the central nervous system and not on the clinical results. The 10 cases of amyotrophic lateral sclerosis that received vitamin E were investigated histopathologically and compared with material from about 40 untreated cases. As will be demonstrated, in many of the treated cases the destruction of myelin sheaths and axis cylinders was less intense than in the untreated cases, while the dense gliosis which is usually present in amyotrophic lateral sclerosis was diminished or almost absent in those that received vitamin E. The anterior horn cells and the nerve cells of the involved bulbar nuclei remained unchanged and showed no signs of reversibility.

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TABLE I

Cases Treated with Vitamin E, Including Alpha-Tocopherol Intramuscularly, Showing Significant Changes in the Involved Pathways

Case no.	Sex	Age	Duration of illness	Treatment with alpha-tocopherol	Bulbar and anterior horn cell disease	Pyramidal tract signs	Histopathologic findings
1	M	64	1 yr., 10 mos.	7½ months	Bulbar symptoms; marked atrophy and fibrillations of muscles, especially in the distal parts of the extremities	Hyperactive deep reflexes; diminished abdominal and cremasteric reflexes; no Babinski or allied signs	Myelin sheath: no demyelinization noted High power examination: slight changes, insular Bielschowsky preparation: slight changes Fat: scarcely any Holzer preparation: very faint gliosis, insular
2	M	60	2 yrs. 7 mos.	1 month and 4 days	Bulbar symptoms; severe atrophy and fibrillations of the muscles of the distal parts of the extremities	Bilateral foot drop; hyperactive deep reflexes with diminished abdominal and cremasteric reflexes, but no other pathologic reflexes	Myelin sheath: faint demyelination with small islands of myelin sheath swelling and destruction, axis cylinders slightly less than in ordinary cases Fat: less fat than normally Holzer preparation: faint gliosis
3	M	44	6 yrs.	4 months and 5 days; treated intensively	Severe atrophy and fibrillations of the muscles of the extremities	Spastic gait with marked hyperreflexia in the upper and depressed in the lower extremities; diminished abdominal and absent cremasteric reflexes	Myelin sheath: scarcely any pallor High power examination: slight disintegration of fibers, insular Bielschowsky preparation: slight disintegration Fat: present, not as much as in ordinary cases Holzer preparation: slight gliosis, insular

4	F	53	1½ yrs.	7 months and 3 weeks	Bulbar symptoms; atrophy and fibrillations of the muscles	Spastic gait; areflexia except for slight knee-jerk; absent abdominal reflexes and presence of a left Babinski sign	Myelin sheath: no visible demyelination High power examination: slight disintegration of fibers, insular Bielschowsky preparation: slight disintegration Fat: very little Holzer preparation: faint gliosis, insular
5	F	70	1 yr., 1 mo.	2 months and 7 days	Bulbar symptoms; atrophy and fibrillations of the muscles of the upper extremities	Hyperactive reflexes with bilateral Hoffmann signs and defective plantar responses	Myelin sheath: very slight demyelination High power examination: slight changes, insular Bielschowsky preparation: slight changes Fat: scarcely any Holzer preparation: faint gliosis, insular
6	M	54	2 yrs.	5 weeks and 3 days; received treatment also on the outside, but could not determine exact amount; this patient showed slight improvement in neurologic symptoms	Bulbar symptoms; atrophy and fibrillations of the muscles of the upper extremities	Generalized hyperreflexia with bilateral Hoffmann signs, patellar and ankle clonus	Myelin sheath: slight pallor of left and slight demyelination of right crossed pyramidal tract High power examination: destruction; however, not as marked as in average case; more marked on the right Fat: same as in ordinary cases Bielschowsky preparation: destruction, but less than in ordinary cases, especially on the right Holzer preparation: moderate gliosis in right crossed pyramidal and very slight on the left

TABLE II

Cases Treated with Vitamin E, Some Inadequately, without Significant Changes in the Involved Pathways

Case no.	Sex	Age	Duration of illness	Treatment with alpha-tocopherol	Bulbar and anterior horn cell disease	Pyramidal tract signs	Histopathologic picture
7	M	53	1 yr., 11 mos.	15 days	Atrophy and fibrillations of muscles	Spastic gait; hyperactive deep reflexes; absent abdominal reflexes and defective plantar responses	Myelin sheath and axis cylinder: glial reaction and deposition of fat not much different from ordinary cases of amyotrophic lateral sclerosis
8	M	50	2½ yrs.	3 mos. 10 days	Bulbar symptoms; atrophy and fibrillations of muscles	Hyperactive deep reflexes with bilateral Hoffmann, Rossolimo and Babinski signs	Myelin sheath: moderate destruction Axis cylinder: moderate destruction Fat: some fat droplets, but not as much as in usual cases Holzer preparation: moderate gliosis
9	F	49	2 yrs.	1 yr.; fairly intensive treatment but with interruptions	Bulbar symptoms; atrophy and fibrillations of the muscles of the upper extremities	Spastic gait; hyperactive reflexes in the upper extremities with bilateral Hoffmann signs but diminished reflexes in the lower extremities with no pathologic reflexes	Myelin sheath: demyelination and disintegration of myelin sheaths Fat: same as average Bielschowsky preparation: about same as average case Holzer preparation: moderate gliosis, slightly less than average
10	F	62	2½ yrs.	7 mos., with some interruptions	Atrophy and fibrillations in the upper extremities	Spastic gait; generalized hyperreflexia with bilateral Hoffmann signs; ankle clonus and absent abdominal reflexes	This case did not differ neuropathologically from untreated cases of amyotrophic lateral sclerosis

Methods of Procedure

Ten cases (Tables I and II) of amyotrophic lateral sclerosis that received vitamin E in the form of ephynal, alpha-tocopherol, a diet rich in vitamin E, thiamine chloride, wholewheat germ oil and bile salts form the basis of this presentation. Two patients received ephynal by mouth without intramuscular injections of alpha-tocopherol. The treatment in the other eight cases conformed to that outlined by Wechsler.[1]

Sections from various cortical areas (where available), internal capsule, peduncles, pons, medulla oblongata and spinal cord were embedded in parlodion and stained by the myelin sheath and cresyl violet methods. Frozen sections were stained by the myelin sheath, Bielschowsky, Sudan III and Holzer methods. For purposes of comparison the gliosis in the untreated cases was designated as dense, while in the treated cases there were gradations which were divided into moderate, slight, or very slight gliosis.

Analysis of Material

Clinical Evaluation

These cases (Tables I and II) present a few clinical facts worth discussing. There were 6 males and 4 females, a distribution which conforms with observations that the incidence of amyotrophic lateral sclerosis is greater in males than females. The ages of these patients were: 2 in the fifth decade, 4 in the sixth, 3 in the seventh, and 1 in the eighth. The duration of the illness in most instances was between 2 and 2½ years, except that one patient (case 3) lived 6 years after the onset of the illness and that three patients (cases 1, 4 and 5) lived 20, 18 and 13 months respectively.

Of the ten patients, two (cases 7 and 8, Table II) received ephynal by mouth; the others also received alpha-tocopherol intramuscularly. Case 7 received the treatment only for a period of 15 days. The others were treated for from 5 weeks to 7½ months. Cases 9 and 10 were treated for 1 year and 7 months respectively, but with interruptions. As will be seen, this may have had some relationship to the histopathologic changes. None of these patients except case 6 showed any improvement in the neurologic symptoms.

The first six treated cases that showed a different histopathologic picture from the usual picture in nontreated cases disclosed severe atrophy and fibrillations of the muscles, especially in the distal parts of the extremities, more so in the upper than in the lower (Table I). The pyramidal tract signs consisted of a generalized hyperreflexia in all, diminished or absent abdominal reflexes in cases 1, 2, 3, 5 and 6 (Table I), Hoffmann's sign in cases 5 and 6, Babinski's or allied signs

only in case 4. These cases ought not to be confused with those of progressive spinal muscular atrophy, a disease of the anterior horn cells without pyramidal tract lesions in which there is widespread atrophy of muscles and absent or markedly diminished deep reflexes. Furthermore, the onset of the illness in progressive spinal muscular atrophy starts at a much earlier age and lasts much longer than in amyotrophic lateral sclerosis, at times from 10 to 20 years. Wilson[11] and others recognized that atrophy of muscles in amyotrophic lateral sclerosis may become so extreme as to abolish or decrease the respective reflexes: "There is as it were a conflict between the respective tone-increasing and tone-reducing influences of supranuclear and nuclear lesions." In an analysis of 36 other cases of amyotrophic lateral sclerosis that came to necropsy, 15 cases with severe demyelination of the pyramidal tracts because of the marked atrophies had diminished or absent reflexes. It should also be emphasized that most of the patients in this presentation, as in the untreated cases of amyotrophic lateral sclerosis, died because of involvement of the bulbar nuclei, a process which rarely occurs in progressive spinal muscular atrophy.

Wilson,[11] who is inclined to place progressive spinal muscular atrophy and amyotrophic lateral sclerosis in the same group, stated:

> "Difficult as it is to reach a firm conclusion, the lesions of the Charcot type [amyotrophic lateral sclerosis] are so characteristic, and, when advanced, so different from those of nuclear amyotrophy [progressive spinal muscular atrophy], that in my opinion the fact outweighs other considerations; they comprise much more than the mere addition of upper to lower motor neuronic disease. While I consider this divergence more striking than the similarities, the occurrence of gradations from one to the other, and also in the direction of 'subacute anterior poliomyelitis,' must be admitted; not all of either kind run true to type. For *descriptive purposes* [not italicized in the original], however, it is best to take the two varieties together, inclusive of intermediate and aberrant clinical forms."

Histopathologic Evaluations

Nerve Cell Changes. The histopathologic changes in the involved nerve cells of the medulla oblongata (usually 10th and 12th nuclei) and anterior horn cells in the spinal cord were the same in the treated as in the untreated cases of amyotrophic lateral sclerosis. This was true both in regard to the number and to the character of the nerve cell changes. The involvement of the bulbar nuclei and the progress of the changes in these nerve cells resulted in death in most of these cases.

Pathway Changes. On the other hand, definite differences were present in the myelin sheaths, axis cylinders, fat and glia.

In cases 1, 2, 3, 4, 5 and 6 (Table I), or 60 per cent of the cases, the pathologic process in the involved pathways of the central nervous

system, especially of the spinal cord, differed entirely from the untreated cases. In these, except case 6, demyelination of the crossed pyramidal tracts in the myelin sheath preparation could hardly be detected with the naked eye (Fig. 1). This was not true in the untreated cases where the demyelination stands out prominently (Fig. 2).

Occasionally a case of amyotrophic lateral sclerosis may show little demyelination in the pyramidal pathways. Two such cases were found among the other 36 cases that came to necropsy. One of these proved to be a case of spastic pseudosclerosis. The diminution of demyelination in the first 6 consecutive cases that were treated certainly could not be a mere coincidence. Furthermore, even the other 4 less adequately treated cases (Table II) showed changes which were slightly less severe than in the ordinary run of amyotrophic lateral sclerosis.

With higher magnification, the involved myelin sheaths, usually grouped in small islands, were fragmented or swollen (Fig. 3). When compared with the changes in the myelin sheaths in untreated cases, it can be stated definitely that this process was not one-tenth as extensive as in the average case of amyotrophic lateral sclerosis (Fig. 4). Occasional swelling of myelin sheaths could be detected also in parts of the pyramidal tracts which did not appear demyelinated (Fig. 5). In case 6, the right pyramidal tract was demyelinated and destruction of myelin sheaths in this pathway was more extensive.

The pathologic process in the axis cylinders, except those of the right pyramidal tract in case 6, was limited essentially to the islands of involved myelin sheaths. Here the changes, although much less intense than in the untreated cases, consisted of swelling, slight fragmentation, bulbous terminations and corkscrew appearance (Fig. 6). Various degrees of swelling of the axis cylinders were noted also in parts of the pyramidal tracts which did not appear demyelinated.

In the Sudan III preparations, the amount of fatty deposits differed in these six treated cases. There were scarcely any fatty deposits in the lateral or anterior pyramidal tracts in cases 1 and 5 (Fig. 9). Cases 2, 3 and 4 had less fat than the untreated cases, while in case 6, the fatty contents did not differ greatly from the untreated cases.

Most of the outstanding changes, however, were noted in the glial tissue. In the untreated cases of amyotrophic lateral sclerosis, there was a dense gliosis in the involved pathways easily detected with the naked eye in the Holzer preparations (Fig. 12). In the six treated cases, except for the right pyramidal tract in case 6, the gliosis could hardly be observed with the naked eye (Fig. 11). With higher magnification, however, small perivascular islands of gliosis (Fig. 13) were noted in the region of the involved myelin sheaths and axis cylinders.

In case 7 (Table II), which was treated for only a short period of time (15 days) with ephynal by mouth, the myelin sheaths, axis cylinders, fat content and glial reaction did not differ from the untreated cases of amyotrophic lateral sclerosis. In cases 8, 9 and 10 (Table II) the destruction of myelin sheaths, axis cylinders and the gliosis were not much different, although slightly less than in the untreated cases. In case 8 the fatty accumulations in the affected pathways were definitely less than in the untreated cases. The glial reaction in these cases was moderate in degree and slightly less intense than in the ordinary cases. In order, however, to be on the conservative side, the changes in these cases were considered to be about the same as those found in untreated cases.

Certain factors may have played a rôle in the histopathologic changes of cases 8, 9 and 10 (Table II). In case 8 the patient received ephynal orally, but did not receive alpha-tocopherol intramuscularly. Cases 9 and 10 were treated for 1 year and 7 months respectively, including intramuscular injections of alpha-tocopherol, but the treatment was interrupted several times. This may have influenced the lack of improvement in the involved pathways. The age, the duration of the illness and the time the treatment was begun in these instances did not differ from the other treated cases.

Factors Influencing the Changes in the Affected Pathways. As was previously demonstrated by me,[12] in untreated cases of amyotrophic lateral sclerosis the disease affects the upper and lower motor neurons. The upper motor neuron can be involved, either at its point of origin in the giant pyramidal cells of Betz or anywhere along its course. The lesion of the upper motor neuron is most pronounced in the spinal cord and is characterized by destruction of the myelin sheaths and axis cylinders, and a dense glial scar. Once the myelin sheaths and axis cylinders in the involved pathways of the central nervous system are completely destroyed, their regeneration is problematic. Regeneration of/or newly formed myelin sheaths in peripheral nerve lesions are known to occur. Recently, Tower [13] avulsed ventral spinal nerve roots from the spinal cord in four cats that were killed after periods of from 4 weeks to 1 year. Evidence of vigorous regeneration on the part of the ventral root fibers was observed at autopsy, either grossly or on microscopic examination, in all of the animals, beginning deep in the rootlet tracts of the cord. As far as I know, this has not been demonstrated in the central nervous system. According to Cajal [14] and others, the failure of regeneration in the central nervous system is due not so much to deficient power of the neurons as to the unfavorable conditions which the central tissues oppose to the growth of the newly formed

neurites. Some observers are under the impression that regeneration of axons in involved pathways of the spinal cord may take place under favorable conditions in a manner similar to regeneration of axons in peripheral nerves. By the new methods of neurofibrillar study, it has been demonstrated that the production of new fibers, clubs, cones and ramified axons may occur in various lesions of man and animals. These findings, while they demonstrate signs of repair comparable in principle with those of the central stump of the peripheral nerves, do not contradict the conception of the impossibility of complete regeneration of axis cylinders in the spinal cord. These later investigations have also demonstrated that after a certain length of time the restoration of the axon in the spinal cord stops, that the axon atrophies and that finally the nerve sprouts break down completely.

On the basis of these investigations, it can hardly be expected that completely destroyed myelin sheaths and axis cylinders in the spinal cord should regenerate. In the six cases of amyotrophic lateral sclerosis adequately treated with vitamin E there was no question that the involved myelin sheaths and axis cylinders were less affected when compared with similar preparations of untreated cases. When I [15] observed similar improvement in the cases of subacute combined degeneration adequately treated with liver, I then postulated that axis cylinders may undergo a reversible reaction provided they are not severely affected and if the patient received early and adequate treatment. Can this explanation also be applied to amyotrophic lateral sclerosis? This cannot be answered as easily as in subacute combined degeneration. There is a possibility in amyotrophic lateral sclerosis, as in subacute combined degeneration, of a reversibility of the reaction if the axis cylinders are not severely destroyed. In other words, damaged but not completely destroyed axis cylinders, under favorable circumstances, may regenerate or be restored nearly to their normal state. This is the only possible explanation that can be offered from the foregoing observations. Under these circumstances, clinical improvement in the neurologic signs and symptoms in my cases should have been expected. This, as already stated, did not occur. How can this discrepancy be explained? The possibility that these patients did not receive adequate quantities of vitamin E and/or were not treated sufficiently early must be considered. In some instances of amyotrophic lateral sclerosis, in addition to the lack of vitamin E which may cause the disease, there is the possibility, as suggested by Wechsler,[1] of another unknown factor, which may play a rôle in the absorption of the vitamin. These are questions which so far have not been settled and cannot be answered with certainty. It is possible that in some instances early and

adequate administration of vitamin E may arrest the process in the involved myelin sheaths and axis cylinders and may restore their function provided they are not completely damaged.

The lessened or almost complete disappearance of fatty deposits in the treated cases can be explained on the basis that vitamin E treatment may have resulted in a decline or cessation of the pathologic process; thus helping toward the removal of the products of disintegration.

The absence of intense gliosis or the regression of the gliosis in the treated cases appears disturbing, for the contrary would be expected in areas of myelin sheath and axis cylinder repair. In a number of cases of subacute combined degeneration studied by me,[15] that received adequate liver therapy, the improvement noted in the affected pathways was accompanied by a dense gliosis. There is a remote possibility that the restoration or reversion to a normal or nearly normal glial reaction as seen in the treated cases of amyotrophic lateral sclerosis is parallel to the observed restoration of the myelin sheaths and axis cylinders. If this should be true, then it may be postulated that the reversibility of reaction took place all along the line, affecting simultaneously and equally, or nearly equally, the damaged myelin sheaths, axis cylinders and glial tissue.

One other disturbing factor in this study was the change in the nerve cells in the involved bulbar nuclei and anterior horns, which did not differ in the treated and untreated cases. Clinically, however, Wechsler [1] observed that the fibrillations and the bulbar signs, especially the recent ones, cleared up, whereas, the pseudobulbar symptoms responded less well or not at all.

There are still many questions regarding the treatment of amyotrophic lateral sclerosis which will have to be answered. While the efficacy of vitamin E therapy in this disease is still problematic, the described histopathologic findings and the clinical (Wechsler [1]) evidence indicate that this form of treatment should be continued experimentally.

Summary and Conclusions

Ten cases of amyotrophic lateral sclerosis were treated with vitamin E and alpha-tocopherol and, except for one, none responded clinically to this form of treatment.

Histopathologically, however, in six of the intensively treated cases the destruction of the myelin sheaths and axis cylinders was found to be much less intense than in the untreated cases. The dense gliosis which is usually present in amyotrophic lateral sclerosis was diminished or almost absent in those that received vitamin E. The lessened myelin

sheath and axis cylinder destruction and the faint gliosis in these instances were perivascular and insular in distribution. In one of these (case 6), the lessened changes were limited to one side of the cord only. The nerve cells of the involved bulbar nuclei and anterior horns remained unchanged and showed no signs of reversibility. The ultimate cause of death was bulbar in nature.

The histopathologic processes in the other four less intensively treated cases, although less extensive, were considered to be about the same as those found in untreated cases.

There is a possibility that vitamin E therapy resulted in a reversal of the reaction of degeneration affecting simultaneously and nearly equally the damaged myelin sheaths, axis cylinders and glia in amyotrophic lateral sclerosis.

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DESCRIPTION OF PLATES

PLATE 106

FIG. 1. Case 1. Lack of visible demyelination in the pyramidal pathways of a case of amyotrophic lateral sclerosis treated with vitamin E. Compare with Figure 2. Myelin sheath stain.

FIG. 2. From a nontreated case, showing extensive demyelination of the crossed pyramidal and left direct pyramidal tracts. Myelin sheath stain.

FIG. 3. Case 1. Insular myelin sheath destruction from a case treated with vitamin E. Compare with Figure 4. Myelin sheath stain. × 240.

FIG. 4. Extensive myelin sheath destruction from an untreated case. Myelin sheath stain. × 240.

FIG. 5. Case 1. Slight disintegration and swelling of single myelin fibers in parts of the pyramidal tracts that appeared uninvolved, from a case of amyotrophic lateral sclerosis that received vitamin E. Myelin sheath stain. × 480.

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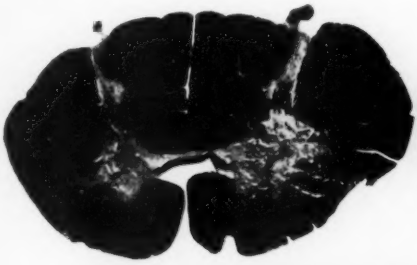

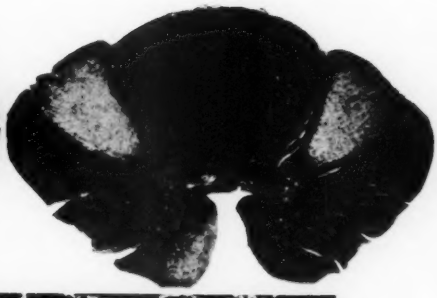

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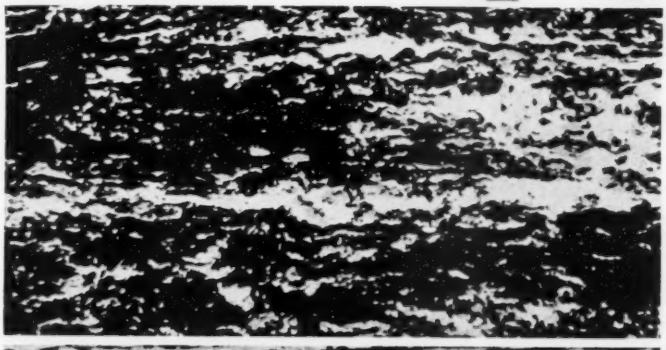

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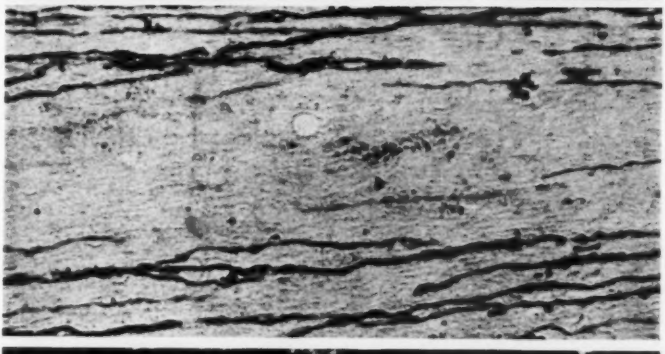

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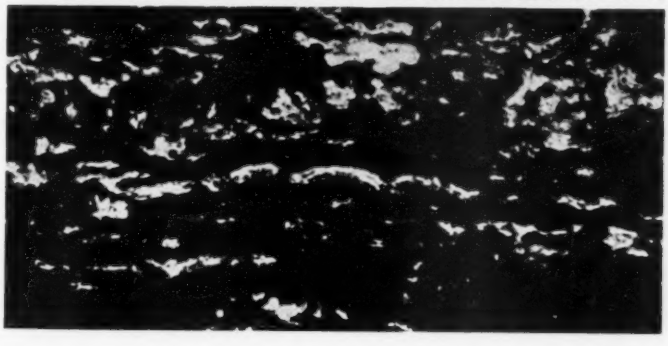


PLATE 107

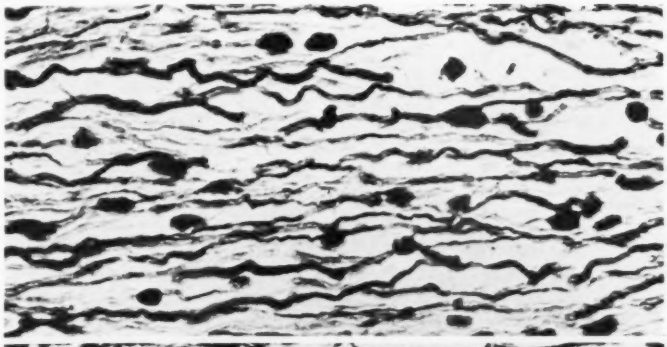
FIG. 6. Case 1. Axis cylinders from the crossed pyramidal tract of a case of amyotrophic lateral sclerosis treated with vitamin E. Compare with Figure 7 from a normal case and Figure 8 from an untreated case of amyotrophic lateral sclerosis. In Figure 6 there is slight diminution in number, swelling and slight tortuosity of axis cylinders when compared with the normal in Figure 7 and the severely diseased, fragmented and swollen axis cylinders of the untreated case of amyotrophic lateral sclerosis in Figure 8. Bielschowsky stain. × 480.

FIG. 7. From a normal control case, for comparison with Figures 6 and 8. Bielschowsky stain. × 480.

FIG. 8. From an untreated case of amyotrophic lateral sclerosis. For comparison with Figures 6 and 7. Bielschowsky stain. × 480.

FIG. 9. Case 1. Almost complete absence of fat in the pyramidal pathways from a case of amyotrophic lateral sclerosis treated with vitamin E. Compare with Figure 10 from an untreated case showing lipoid deposits throughout and in the perivascular spaces. Sudan III stain. × 100.

FIG. 10. From an untreated case of amyotrophic lateral sclerosis, for comparison with Figure 9. Sudan III stain. × 100.



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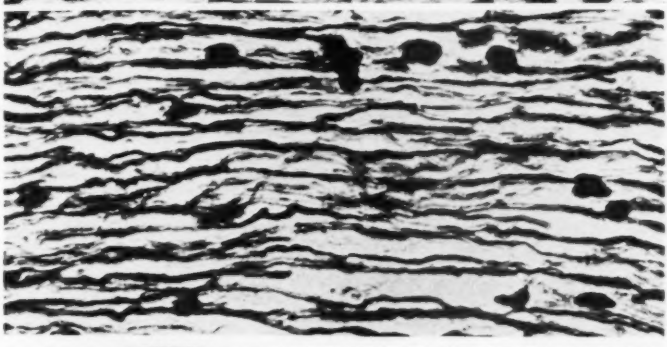

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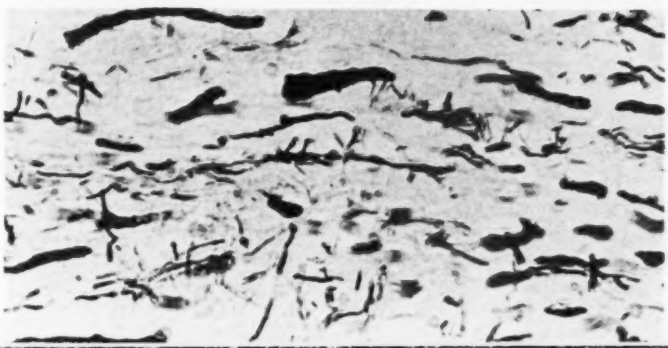

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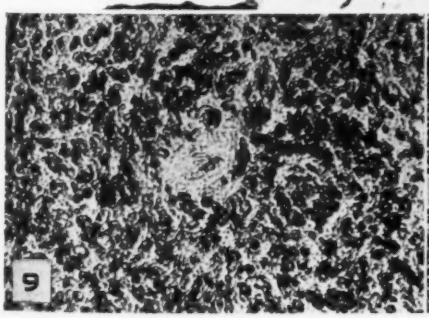

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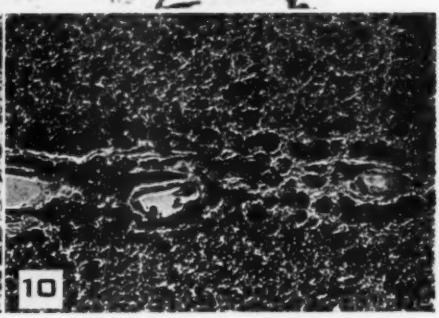

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PLATE 108

FIG. 11. Case 1. Lack of gliosis in the pyramidal tracts from a case of amyotrophic lateral sclerosis treated with vitamin E. Compare with Figure 12 from an untreated case of amyotrophic lateral sclerosis showing dense gliosis in the crossed pyramidal tracts. Holzer stain.

FIG. 12. From an untreated case of amyotrophic lateral sclerosis, for comparison with Figure 11. Holzer stain.

FIG. 13. Case 1. Slight insular gliosis from a treated case of amyotrophic lateral sclerosis. Compare with Figure 14 from an untreated case and Figure 15 from a case of descending degeneration. In Figures 14 and 15 the gliosis is dense; more so, however, in the case of descending demyelination. Holzer stain. × 200.

FIG. 14. From an untreated case of amyotrophic lateral sclerosis, for comparison with Figure 13. Holzer stain. × 200.

FIG. 15. From a case of descending degeneration, for comparison with Figures 13 and 14. Holzer stain. × 200.

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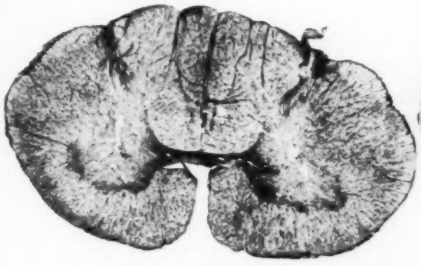

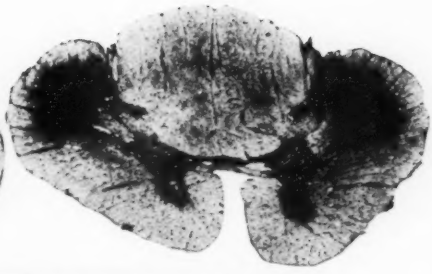

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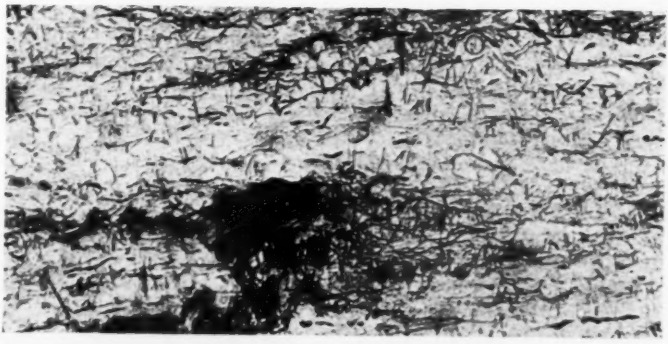

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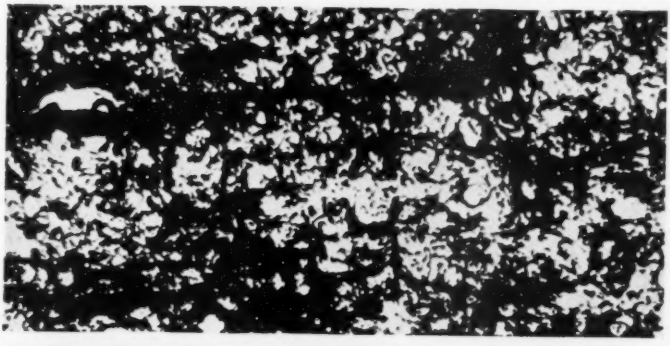
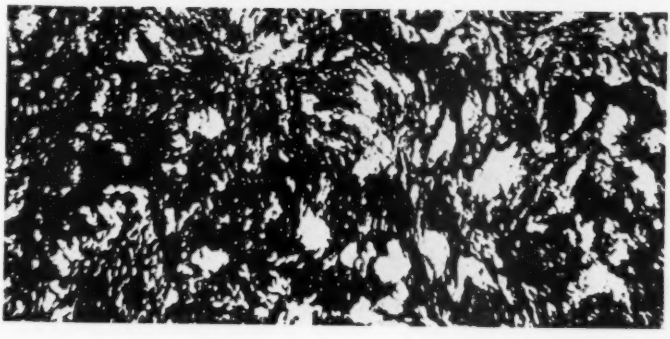
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